

The University of Chicago

STUDIES ON HETEROPLASTIC
TRANSPLANTATION IN TRICLADS. I. CE-
PHALIC GRAFTS BETWEEN *EUPLANARIA*
DOROTOCEPHALA AND *E. TIGRINA*

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOÖLOGY

1937
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By
JAMES ALBERT MILLER, JR.

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STUDIES ON HETEROPLASTIC TRANSPLANTATION IN TRICLADS
I. CEPHALIC GRAFTS BETWEEN EUPLANARIA
DOROTOCEPHALA AND E. TIGRINA¹

(Ninety-eight figures)

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IT IS commonly recognized by experimenters that by the production of abnormal relationships between parts it is often possible to isolate some of the factors which operate in the development and maintenance of the normal characteristics of organisms. The large volume of work on extirpation, explantation, and transplantation is evidence of the possibilities of this type of experimental procedure.

In the study reported here, interspecific transplantation among triclad flatworms was selected as a method of studying certain phases of "dominance," a phenomenon which has been recognized in the zoölogy laboratory of the University of Chicago for the last quarter of a century (Child, 1911a) and has recently come to the attention of embryologists in various parts of the world (Huxley and de Beer, 1934; Huxley, 1935; Weiss, 1935; Strelin and Trifonova, 1935). For a brief review of the characteristics of the dominant region of the simpler invertebrates see J. A. Miller (1937).

Most of the earlier workers on transplantation in the lower invertebrates have been concerned with problems other than those attacked here. However, numerous instances of dominance may be gleaned from their data. Among coelenterates, Browne (1909) found that small pieces of the hypostome region of *Hydra* will determine a new apical region in the host and modify its polarity. Rand, Bovard, and Minnich (1926) confirmed these results and demonstrated the "inhibitory dominance" of such a graft. Mutz (1930), using *Pelmatohydra* and *Hydra*, showed that the effects are not species specific. Burt (1934) found a gradient in the ability of grafted rings of tissue from different levels of *Pelmatohydra oligactis* to dominate the host. Child (1929b, 1932) studied dominance in *Corymorpha* by means of grafting. In this form pieces from all levels may induce a new hydranth and stalk, but distal pieces are most potent. It is not even necessary to implant any tissue, for laceration of the edges of an incision is sufficient stimulus to cause the cells in the vicinity to produce a hydranth (Child, 1927, 1929b).

Among the flatworms, Moretti (1911), using *Planaria torva*, found that only grafts of the head could persist and maintain the autonomic movements. He figured and described cases of inhibition of regeneration after removal of the host head and of induction of a pharynx by grafts of the cephalic region but did not discuss them. Rand and Browne (1926) reported four cases in *Euplanaria tigrina* (syn. *Planaria maculata* [Kenk, 1935]) in which there was no regeneration of the host head after its removal close to a cephalic graft. Gebhardt (1926) reported a few cases in *Planaria lugubris* in which there were outgrowths of the body as a result of grafting. Goetsch (1926, 1929) found that a graft

¹ The writer wishes to express very great appreciation for the interest and inspiration of Professor C. M. Child in this work, as well as for proposing this line of investigation and for helpful suggestions during its progress.



of a regenerating head could prevent the development of a host head and eventually take its place. When a small portion of the host was isolated with the graft, it not only prevented development of head and tail but seemed to grow at the expense of the host. Santos (1929, 1931) has made the most complete study of dominance by the grafting technique. He found that ganglionic grafts of *Euplanaria dorocephala* in prepharyngeal regions could induce the development of postcephalic structures. In pharyngeal regions they might induce a pharynx, and in postpharyngeal regions two pharynges might be induced with the one anterior to the graft in reversed polarity. The indications were that the ability of the graft to inhibit the regeneration of a host head depends upon the level of implantation. These effects were not species-specific, for heteroplastic transplants between *E. dorocephala* and *E. tigrina* gave the same results. Grafts other than from the head did not possess this reorganizing power.

Steinmann (1933), using *Dendrocoelum lacteum*, reported a case in which a ganglionic graft placed in the tail developed two small pharynges and more or less complete sets of reproductive organs. Finally, Okada and Sugino (1934) reported cases of dominance of host by graft in the Japanese *Planaria gonocephala*, in which not only pieces containing the cephalic ganglia but also those from between the head and pharynx could induce pharynges in postpharyngeal regions of the host.

In the experiments reported here heteroplastic transplants were used as a means of distinguishing between implant and host in order to secure more exact data on the regeneration of the graft, the ability of grafts from various levels of the donor to maintain themselves, the differences in inductive capacity of grafts from various levels, and the differences in resistance of various host levels to the domination of grafts.

MATERIAL

The animals used in these experiments were *E. dorocephala*, collected in spring-fed streams near Rockford and Cary, Illinois, and Valparaiso, Indiana; *E. tigrina* (Kenk, 1935; syn. *P. maculata*) from Valparaiso; and *E. tigrina novangliae*² from a pond in Falmouth, Massachusetts. These three forms are closely related anatomically and proved well adapted to the requirements of the experiments reported here. Takes were readily secured; yet there were sufficient external differences to distinguish between graft and host tissues, a fact which permitted accurate data on the fate of the graft and on its inductive capacity. Most of the transplantations were between *E. dorocephala* and *E. tigrina novangliae*.

The external differences by which these three forms may be distinguished are listed below. They consist of differences in size, shape, pigmentation, and appearance of the digestive tract and of the photoreceptors. *Euplanaria dorocephala* (Pl. I, Fig. 1) is the longest of the three; *E. tigrina* (Pl. I, Fig. 2), the shortest; and *E. tigrina novangliae* (Pl. I, Fig. 3), the widest. There are consistent differences in shape of head. The head of *E. dorocephala* is pointed with long "auricles" and a slight constriction in the "neck region"; that of *E. tigrina* is blunt with short and very wide "auricles" and with very little constriction in the "neck region."

With respect to pigmentation the three forms are quite distinct. The pigment of *E. dorocephala*, almost black in nature and dark to light brown when kept in the labora-

² *Euplanaria tigrina* and *E. tigrina novangliae* are so closely related that Kenk (1935, p. 85) stated that "the copulatory organs afforded no means of discriminating between them" but that on the basis of the external differences they may possibly be subspecies.

tory, is distributed over the surface of the animals in small, dark granules against a brown or tan background. The ventral surface is a grayish hue because of the lighter background than that on the dorsal surface. The dorsal surface of *E. tigrina* (as represented at Valparaiso, Indiana) is finely maculated with yellowish-orange pigmented areas against a tan background. The ventral surface of this form is white with gray pigment areas, giving a light-gray effect. The coloration of *E. tigrina novangliae* is very variable; therefore, only one type was used in these experiments. The dorsal surface of this type is characterized by large pigmented areas, some of which are light and some of which are dark brown, interspersed with occasional colorless areas, and by a mid-dorsal light stripe, along each side of which there is an irregular row of darker spots. The ventral surface of these planarians is much darker than that of *E. tigrina maculata* and has a brownish tinge.

The photoreceptors of the three can be distinguished by the differences in the size and shape of the unpigmented areas. Those of *E. dorocephala* are elongated and set in such a fashion that the anterior parts are closer together than the posterior. Those of *E. tigrina* are very large and are placed with their axes parallel to each other. The crescentic pigmented areas of the photoreceptors are completely visible in this form. In the case of *E. tigrina novangliae* the unpigmented areas are less perfectly oval, are smaller, and do not completely expose the pigmented areas.

The branches of the digestive tract of living planarians may be readily observed by allowing the animals to feed on clotted or dried blood. Those of *E. dorocephala* are characterized by extensive anastomoses and by the formation of accessory longitudinal trunks in connection with both anterior and posterior rami of the intestine (Hyman, 1925). On the other hand, the branches of the intestine of *E. tigrina* are more regular and do not develop the anastomoses and accessory trunks such as are characteristic of the other species.

Preliminary transplants were made according to the method described by Santos (1931); however, it was found that the degree of anesthesia could be more easily controlled and that recovery was more rapid when a physical agent (low temperature) was used instead of a chemical one (chloretone). Likewise, it became apparent that the use of rectangular grafts containing the same volume of tissue as the triangular ones used by Santos resulted in a higher percentage of "takes," since incisions of this shape had less tendency to cause the host to tear apart at the angles. With these modifications, very high percentages of unions were obtained, usually between 75 and 100 per cent.

PROCEDURE

Except in cases in which grafts were made in the cephalic region the prospective hosts were decapitated from 2 to 4 hours before operation in order to reduce their activity. The recipient was placed, together with a small amount of well water, on a clean, grease-free glass square resting on a pan of ice from a refrigerator. When the host was sufficiently anesthetized, a rectangular window was removed with the aid of two very sharp half-spear dissecting needles under a low-power (55-mm. objective) binocular microscope. Because of the strong contraction of the cut surfaces, it was found necessary to make the graft considerably smaller than the window into which it was to be placed. It was placed in the desired orientation, and the excess water drained off the plate. Then a Petrie dish with vaselined edges was inverted over the plate, making an airtight chamber. This was placed in the dark for 8-12 hours, after which the "takes" were transferred

to finger bowls with approximately 200 cc. of well water, and were measured and recorded. At this time controls from the same stock were cut with windows as nearly like those of experimentals as possible, but no graft was inserted. Both experimental and control animals were fed beef liver or dried blood, and the water was changed twice a week. Subsequent observations were made at weekly intervals. Certain of the specimens were killed in a 0.5 per cent solution of HNO_3 and fixed in a saturated solution of HgCl_2 in a 0.6 per cent solution of NaCl (Willier, Hyman, and Rifenburgh, 1925). A report of the histological study of these grafts will be made at a future date.

Large specimens of the three forms were used. Those of *E. dorotocephala* were between 17 and 22 mm. in length; those of *E. tigrina* between 11 and 14 mm.; and those of *E. tigrina novangliae* between 11 and 16 mm. The grafts usually ranged in size from 0.8×1.0 mm. to 1.1×1.3 mm. The few that were smaller occasionally were resorbed, while in larger ones the frequency of takes was low.

Three regions of the body were selected for study: the head region, which contains a concentration of nervous elements; the pharynx, which contains highly differentiated muscular and nervous elements; and the tail, which is the actively growing but least differentiated portion of the animal. Grafts were taken from each of these regions of the donor and transplanted into each of three regions of the hosts. All possible combinations of dorsal and ventral with anterior and posterior surfaces of graft and host were made.

In order to make the results comparable, grafts were cut from, and implanted into, as nearly the same levels as possible. All cephalic grafts and windows in the hosts were cut with the anterior surface just in front of the unpigmented areas of the eyes; pharyngeal grafts and windows were cut to include the muscles attaching the proximal end of the pharynx to the body wall. Tail grafts and windows were cut from the most posterior tips of the tail. They were made slightly wider at the anterior end to include as much of the growing region as possible. In addition, 20 cephalic grafts were implanted just behind the eyes for comparative purposes. In this report only the results of grafts from the cephalic region will be discussed.

Since neither the head nor tail levels of this study corresponded exactly to the regions used by Santos (1929, 1931), a different terminology has been employed in referring to them. The head grafts in this study did not contain the entire ganglionic region and therefore are called "cephalic," rather than "ganglionic," grafts. Similarly, the most posterior level used is designated as the "tail" region, and grafts from it as "tail grafts," because they were located more posteriorly than the postpharyngeal level of Santos. The "tail" represents the more posterior levels of the posterior zooid region. The terminology used in describing the orientation of the grafts is a modification of that introduced by Harrison (1921) and widely used in amphibian experiments. When the dorsoventral axes of graft and host coincided, the implant is referred to as a "dorso-dorsal"; when reversed, it is called a "dorsoventral." When the anteroposterior axes coincided, the graft is an "anteroanterior"; when reversed, an "anteroposterior."

Records were kept on mimeographed sheets with columns for date, measurements (graft length and width; host length and width), drawings, and notes. The notes consisted of observations on the extent of union between graft and host, the development of the transplant, the behavior of host and graft, and feeding responses. In the more recent experiments the weekly drawings were supplemented by photographs taken with a Leica camera and a 1:1 copying attachment. In most cases the outlines of the digestive tract were recorded after feeding the specimens dried blood.

EXPERIMENTAL

GENERAL OBSERVATIONS ON GRAFTS AND CONTROLS

A total of 412 cases of successful transplantation of cephalic pieces were studied. Of these, 105 were placed in the head region, 20 in the anterior prepharyngeal region, 113 in the pharyngeal region, and 174 in the tail. No species differences in inductive potency of grafts or in resistance to induction by the host, as indicated by Santos (1931), were noted. For this reason, and in order to avoid excessive length in the experimental section, no distinctions between species will be made in the descriptions.

Takes.—Although in all types of transplants reported here 100 per cent takes were obtained upon occasions, in general union occurred more commonly with *E. tigrina* as host than with *E. dorotocephala*. Of the three host regions employed, the tail gave the lowest percentage of takes and the head the highest. Of the four cut surfaces of the graft, the anterior united with the host the least frequently and the posterior the most.

Behavior.—Implants in the head in dorsodorsal anteroanterior orientation which united by all surfaces often became immediately incorporated into the host, and normal behavior of the host became re-established within 12 hours after operation. When possessing a free surface or placed in other orientations, grafts in the head usually maintained their own individuality but did not extend their dominance appreciably into the surrounding tissue. On the other hand, when placed in the pharynx region, cephalic grafts controlled the movements of more posterior portions of the host but were unable to gain ascendancy over the more anterior regions. The orientation and degree of union did not greatly affect the ability of grafts to control host movements when placed in the pharynx or tail. Cephalic grafts placed in the tail region controlled the movements of the host both anterior and posterior to the level of implantation.

When two or more heads were present in the same animal, as was the case in most of the experimental animals, considerable difficulty was experienced in inducing the worms to feed. The stimulation by food was considerably greater in these than in normal worms; and, though they would crawl over the food again and again, the pharynx was unable to start feeding before being dragged off by the very active heads. In some cases it was possible to induce these animals to feed by covering the containers with a black cloth. In the absence of stimulation of the photoreceptors the activity of the heads was sufficiently reduced to permit the institution of feeding-responses by the pharynx.

Regeneration.—In all levels a free surface was necessary for regeneration of the normal head shape by the implant. The free surface likewise determined its polarity, with modifications up to 180° not uncommon.

Union of the digestive tracts.—Union of the intestinal diverticula of the graft with those of the host was necessary for the persistence, as well as for the growth, of the graft. However, even in starving grafts, regeneration of lost parts from a free surface occurred, and the ability to induce host structures was not appreciably reduced.

Disintegration of host tissues in the neighborhood of implants.—In certain grafts in the head, prepharyngeal, and pharyngeal regions disintegration of tissue anterior to the level of implantation occurred. In most of these cases no food was observed, after feedings, in the regions where disintegration occurred later. In the rest, the digestive tracts in the affected regions reunited with small side branches and often received little or no food. In all, the typical changes which have been observed in starving planarians (including disintegration of the head) were noted. Accordingly, it is suggested that the disintegration of the heads in these cases might have been caused by starvation.

In many of the cases in which union of graft and host was only partial, sooner or later the host tissue on one side of the graft disintegrated and the implant which had projected dorsally from the host came to lie in the same plane as the host. Disintegration of this localized area is thought to have occurred as a result of the activities of the graft, which, in applying itself to the substrate, constricted the host tissue lateral to it. Whether disintegration was due to mere mechanical pressure or to some secondary effect of the constriction, such as interference with food supply and waste-removal, cannot be said.

Controls.—In the controls, in which a window was made but no graft was inserted, the cut surfaces united within a few hours after the operation. In the head the anterior and posterior surfaces usually united, while in the pharyngeal and tail regions it was the lateral surfaces of the windows which fused. Usually within 2 weeks new tissue had filled in the injury completely, and the structures which had been removed were replaced. There were very few exceptions to this procedure; they will be treated in their appropriate places in the sections which follow.

GRAFTS IN THE HEAD REGION (105 CASES)

Dorsodorsal anteroanterior orientation of graft (24 cases).—The behavior of cephalic grafts implanted in the head region depended principally upon their orientation and the degree of union with the host. When both dorsoventral and anteroposterior axes coincided, grafts and hosts united along all surfaces (22 of 24 cases). Within 12 hours after operation the experimental animals were observed to crawl normally and react normally to food; whereas in the case of the controls, normal locomotion and feeding responses did not appear until regeneration had proceeded as far as the development of approximately the normal head shape and photoreceptors, a week or more later. In almost all cases the photoreceptors of the implants happened to lie in the eye-level of the host. In these, proliferation of host tissue was at a minimum and no host eyes³ were reconstituted (Figs. 4, 72). In contrast to the experimentals the control planarians in every case regenerated the tissue removed, including the two photoreceptors. In 3 cases supernumerary eyes were developed (Figs. 5, 6).

When the photoreceptors of the implants did not happen to lie in the eye-level of the host, one or more eyes were reconstituted by the host (Fig. 7).

In 4 cases the tip of the host head disintegrated, leaving the anterior portion of the graft exposed. These resulted in a chimaera-type head of which the mesial part was of one species and the "auricles" and sides were of the other (Fig. 8).

In all grafts which were kept 3 months or more, there was a gradual replacement of graft epidermis and of graft photoreceptors by those of the host. Whether other tissues were involved is not known. This replacement, which was observed not only in grafts in the head region but also in those in the other two levels used in this study, proceeded as follows. Usually within 1 week after implantation the edges of the transplant became less clearly defined and a ring of unpigmented tissue was observed surrounding the graft. This ring of new tissue (regenerating tissue in these planarians does not become heavily pigmented for 2 weeks or more) was observed gradually to encroach upon the graft during the next 6–8 weeks until all that could be seen of the implant was a few darker pigment spots between the photoreceptors. Eventually they, too, disappeared. Meanwhile, pigment spots characteristic of the host species developed around the

³ Strictly speaking, planarians possess photoreceptors and not eyes, since they have no apparatus for focusing an image upon the photosensitive cells. However, in this report "eye" is used in its less exact sense as a synonym of photoreceptor.

periphery of the new tissue, showing that the cells replacing those of the graft were of host and not of graft origin. It was not possible to follow the process of replacement of the photoreceptors with the same degree of assurance as in the case of the epidermis. However, the change in shape of the unpigmented areas from that characteristic of the graft to that of the host seemed to occur following the depigmentation of the surrounding tissue. The black crescentic pigment areas of the photoreceptors also changed shape gradually but by such imperceptible stages that in most cases the process could not be followed. In 3 cases the black pigment areas of the photoreceptors of the graft were observed to decrease in size gradually, and later a new pigment spot appeared in the unpigmented area of each eye. This increased in size until it fused with that of the graft. Whether this represents the usual situation is not known.

GRAFTS IN THE HEAD REGION

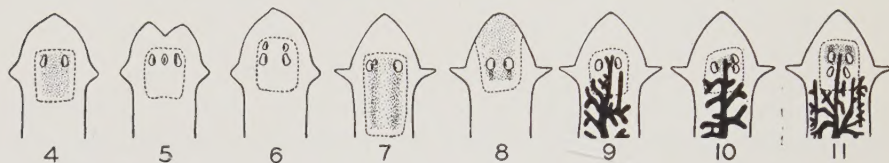


FIG. 4.—*E. dorotocephala* into *E. tigrina novangliae*. DDAA.* Union complete. 31 days. Epithelia of implant were eventually replaced by host tissue.

FIGS. 5 AND 6.—*E. tigrina novangliae*. Controls 31 days. FIG. 5.—Three photoreceptors have developed in place of the two which were removed. FIG. 6.—Four photoreceptors have developed in the new tissue.

FIGS. 7 AND 8.—*E. tigrina novangliae* into *E. dorotocephala*. DDAA. 35 days. FIG. 7.—Union complete. Large graft (1.4 × 0.8 mm.). Eye appeared in new tissue left of implant 2 weeks after grafting. Left graft eye became reduced in size and subsequently fused with it. FIG. 8.—Anterior edge of implant failed to unite with host. Host head disintegrated anterior to implant, resulting in chimaera-type head.

FIGS. 9, 10, AND 11.—*E. tigrina novangliae* into *E. dorotocephala*. DDAP. Union complete. FIG. 9.—35 days. A small photoreceptor appeared anterior to right graft eye 1 week after implantation and disappeared 2 weeks later. Graft epithelia completely replaced by new tissue. Note that digestive tract extends farther anteriorly than normally. FIG. 10.—14 days. One eye appeared posterior to left graft eye 1 week after implantation. Four weeks later new tissue had completely replaced graft epithelia. FIG. 11.—28 days after operation. Two eyes have developed in new tissue posterior to implant.

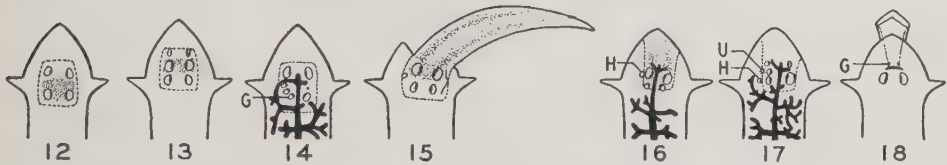
Dorsodorsal anteroposterior orientation of graft (64 cases).—Cephalic transplants placed in the head region with their anteroposterior axes reversed were some of the most interesting of all the types studied. The percentage of cases of complete union (42 per cent) was less in this class of grafts than in those in which the anteroposterior axes coincided. When reversed, the relatively thick posterior surface of the graft was in contact with the thin anterior surface of the host window, and vice versa. It is thought that this was chiefly responsible for the proportionally fewer cases of complete union in these grafts than in the class just reported.

In cases of complete union the grafts showed little independent activity, and the proliferation of tissue resulting from the implantation was so slight that in 5 cases no outgrowth was noted and in the rest it was very small. In all but 4 cases more than one

* In these and subsequent figures the following conventions are used: graft tissue stippled where distinguishable, approximate extent of new tissue indicated by broken lines, invaginated portions by alternating dots and dashes, main intestinal branches by solid black. Time after operation given in days. Orientation of implants: DDAA=dorsodorsal, anteroanterior; DDAP=dorsodorsal, anteroposterior; DVAA=dorsoventral, anteroanterior; DVAP=dorsoventral, anteroposterior.

photoreceptor developed. Of the exceptional cases, 1 developed a very small eye which later regressed (Fig. 9), and the other 3 cases regulated a permanent photoreceptor (Fig. 10). The usual result was the development of one or more photoreceptors in the new host tissue either anterior or posterior to each eye of the graft. In 3 cases one supernumerary eye developed anterior to each eye of the graft, and in 3 two eyes developed posterior to those of the graft (Figs. 11 and 12). There were 3 cases in which four eyes developed, two of them anterior and two posterior to those of the implant (Fig. 13). In other cases the supernumerary photoreceptors were not as regularly arranged, although they never appeared very far from the region of the mesiolateral axis at which eyes are found normally (Fig. 14). It was noted that the supernumerary eyes usually appeared considerably later than did the regenerating eyes of the controls, with a delay of 2 or 3 weeks not uncommon.

GRAFTS IN THE HEAD REGION



FIGS. 12, 14, 15, 16, AND 17.—*E. tigrina* into *E. dorochocephala*. FIGS. 13 AND 18.—*E. tigrina novangliae* into *E. dorochocephala*. In all but Figs. 15 and 18 union was complete. DDAP.

FIG. 12.—35 days. Two eyes have developed in new tissue posterior to implant.

FIG. 13.—28 days. Two eyes have developed in new tissue anterior to implant and two more posterior.

FIG. 14.—56 days. Two eyes appeared anterior to implant 1 week after implantation. Three more developed later in new tissue between graft and host eyes. Left graft eye (G) has become reduced in size.

FIG. 15.—Host disintegrated anterior to implant. 42 days. Tail-like outgrowth has regenerated from free graft surface. Two eyes developed in new tissue opposite graft eyes 1 week after implantation. Unpigmented area on left and projection on right developed later.

FIGS. 16 AND 17.—7 and 49 days, respectively. FIG. 16.—Unpigmented portion of left host eye (H) not completely removed. Two eyes have appeared posterior to graft. FIG. 17.—Graft epithelia completely replaced by new tissue. Graft eyes have fused with two supernumerary eyes shown in Fig. 16. Five additional eyes developed anterior to graft eyes but have been reduced by fusions to three. Unpigmented projection (U) had developed to left of implant.

FIG. 18.—35 days. Posterior surface of graft failed to unite with host. Resulting tubular outgrowth lined with ventral epithelium, projects from ventral surface of host. Pigment areas of graft eyes (G) visible. Two eyes present in new tissue posterior to implant.

A comparison of the conditions of the digestive tracts of the experimental and control animals showed that in the former, as a result of reversing the anteroposterior axis of the graft, the intestine extended into the head of the compound well anterior to the photoreceptors of the implant. In the controls the most anterior branch ended between the regenerated eyes. Usually when there were side branches of the digestive tract anterior to the eyes of the graft, supernumerary eyes developed anterior to those of the implant. However, the correlation between diverticula and number of eyes was not sufficiently close to be interpreted as the determination of the latter by the former. It seems probable that farther anterior extension of the digestive tract in these specimens merely furnishes a greater supply of nourishment to the more anterior levels of the head and allows the development of eyes which might otherwise suffer from competition with the graft.

The presence of a free surface in cases of incomplete union resulted in greater activity on the part of the implant and considerably greater proliferation of tissue as compared

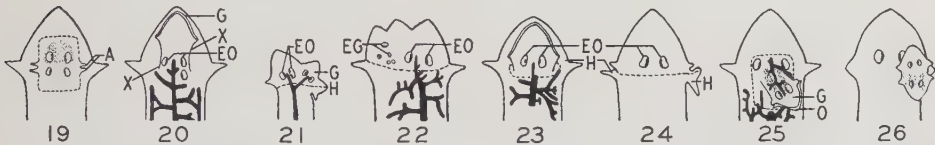
with cases of complete union. In 3 cases the host tissue anterior to the implant disintegrated within a day or two after grafting, leaving the posterior surface of the graft free. In two of these the implant retained its original polarity and showed almost continuous activity, contracting and extending entirely independently of the movement of the host. An outgrowth resembling a tail developed from the free surface of the implant, and two eyes formed in the regenerating host tissue directly behind and opposite those of the graft (Fig. 15). In the third case fusion of graft and host occurred without any appreciable proliferation of new tissue, as was the case in grafts in which both dorsoventral and anteroposterior axes coincided. However, within a week two supernumerary eyes had appeared posterior to those of the implant (Fig. 16). During the next 3 weeks five more developed anterior to them, and in addition there was by this time an unpigmented projection suggestive of an abortive cephalic lobe to the left of the graft. In the ensuing 4 weeks the eyes of the graft fused with the two supernumerary eyes that were posterior to them, and fusions were occurring in the eyes which had developed anterior to the graft (Fig. 17). Thus, even though the implant had its polarity reversed as a result of its position, nevertheless it produced a marked effect upon the host head.

In 19 cases a part of one surface of the graft failed to unite with the host. From this free surface and the cut surface of the host developed a tubular outgrowth around which later appeared supernumerary eyes. Usually it was possible to determine by the position of the developing eyes that the free surface was anterior, even though in some cases it developed from the lateral or posterior surface of the graft. In most cases the outgrowth extended from the upper surface of the host and was surrounded by dorsal epithelium with the ventral surface on the inside of the tube. In one case, however, it extended ventrally and the dorsal surface was inside (Fig. 18). Figure 19 shows a case in which there were two free surfaces—one a small portion of the left, and the other a smaller portion of the right side of the implant. The two free surfaces developed outgrowths resembling cephalic lobes with their unpigmented sensory areas, and a second pair of photoreceptors developed in the regenerating host tissue opposite the anterior surface of the graft.

In 8 cases the entire anterior surface of the implant remained free. In these the anteroposterior axes of the transplants were not affected. Each graft regenerated a normal or nearly normal head, and the host developed a second head from the free edge of the window directly opposite the graft. This grew rapidly until it reached the size of the head of the implant, and during all subsequent observations both remained the same size except when conditions were altered. The presence in the ganglionic region of the host of an *active* graft with its anteroposterior axis reversed was sufficient, in all cases except one, to inhibit completely the development of eyes anterior to it. It appears that grafts that were able to escape the domination of the host and retain their own individuality, because of the free anterior surface, so altered conditions in the head of the host that the physiological level at which eyes are produced did not develop. On the other hand, it should be noted that, even when an implant lacking a free surface became dominated by the host to the extent of having its polarity reversed, it still exerted a modifying influence on the host, extending the physiological level at which eyes could develop over a much wider area than is normally found in these species. In this connection it might be recalled that Abeloos (1930) reports finding specimens in his stocks of *P. gonocephala* with supernumerary eyes usually arranged, as was the case in these experiments, in pairs extending toward the anterior margin of the head. He listed other reports of

supernumerary eyes (Janichen, 1897; Lang, 1913) and attributed them to senescent conditions, since they usually appeared in the largest specimens. However, Child (1921) has shown that susceptibility to inhibiting agents acting during head regeneration, as indicated by modifications of head-form and position or absence of eyes, decreases from the median region laterally. This indicates a higher gradient level in median than in lateral regions. Consequently, the hypothesis that the degenerative changes associated with senescence should cause an increase in the breadth of the area in which eyes are found seems improbable. In the present studies it was noted repeatedly that, when

GRAFTS IN THE HEAD REGION



FIGS. 19-22.—*E. tigrina* into *E. dorocephala*. FIGS. 23-25.—*E. tigrina novangliae* into *E. dorocephala*. FIG. 26.—*E. dorocephala* into *E. tigrina novangliae*. In all but Figs. 19 and 26 anterior surface of graft failed to unite with host. Orientation of all DDAP.

FIG. 19.—21 days. From each lateral free surface two auricle-like structures (*A*) developed. Two eyes have appeared posterior to graft.

FIGS. 20 AND 21.—28 and 56 days, respectively. FIG. 20.—Tubular outgrowth, of which the graft forms the anterior half, has developed. Three eyes illustrated (*EO*) are in host portion of outgrowth. Ventral surface of implant (*G*) visible just behind anterior edge of outgrowth. Disintegration of anterior part of host to *X-X* and of region of union between two components of tubular outgrowth resulted in condition shown in Fig. 21. Right remnant of host head (*H*) and graft (*G*) form right component, and host portion of outgrowth forms the other. Unpigmented areas of right eyes have fused. Graft epithelia have been completely replaced by host tissue.

FIG. 22.—28 days. Tubular outgrowth with two eyes (*EO*) developed and, by disintegration of more anterior parts of host and region of union on left, produced a wide, flat composite head. Double unpigmented area appeared at line of junction between graft and host. Eye has appeared beside right graft eye. Graft epithelia completely replaced by new tissue.

FIGS. 23 AND 24.—7 and 28 days, respectively. FIG. 23.—Graft (*G*) almost entirely hidden behind tubular outgrowth of new tissue in which are two eyes (*EO*). Digestive tract did not reach host or graft head. FIG. 24.—Host and graft heads disintegrated (host by fourteenth day after implantation, graft by twenty-first day), leaving only host portion of outgrowth and auricles of original head (*H*).

FIG. 25.—14 days. Host portion of tubular outgrowth (*O*) lies behind graft (*G*). In contrast to Fig. 23 graft and host digestive tracts united. During 5 weeks following, before it was fixed, graft and host portions of outgrowth continued to increase in size.

FIG. 26.—Graft united to host by posterior part of ventral surface only. 14 days. Digestive tracts of graft and host failed to unite. Head with reversed polarity developed from posterior edge of graft. Graft decreased in size rapidly and separated from host 3 or 4 days after this drawing was made.

supernumerary eyes appeared, they developed in the new tissue during the period of active regeneration of the host, and that later there was a tendency for the eyes to regress and be resorbed.

In 3 cases the portion of the host anterior to the graft was devoid of intestinal branches. During 4 or 5 weeks these portions gradually decreased in size, lost their pigmentation, and finally disintegrated. The graft head and that of the host which had developed behind the graft now became the functional heads of the compound (Figs. 20 and 21). In 2 of the 3 cases the digestive tract of the graft did not unite with the host,

and in these cases the graft also regressed. In 1 case it became an appendage on the left side of the second host head (Fig. 22); while in the other it, too, disintegrated, leaving the second host head at the anterior end of the host (Figs. 23 and 24).

Figure 25 shows the result when both the graft and the anterior portion of the host re-established connections with diverticula from the host intestine. In this case the graft and the head behind it grew until attaining a size practically equivalent to that of the original host head. A postcephalic outgrowth of tissue similar to that described by Santos (1931) was induced by both the graft and the host head.

In 2 instances the amount of union between implant and host was very slight. In both, even though the grafts rapidly decreased in size and gave evidence of starvation from the first, they regenerated "auricles" and the triangular head shape which is normal for the species used. In 1 case (Fig. 26) the graft developed a second head on its free posterior surface, reminding one of the findings of Morgan (1903) and Child (1911*b*) that very short transverse sections of planarians sometimes develop bipolar forms.

Dorsoventral anteroanterior orientation of graft (16 cases).—When transplants were made in the head region with the dorsoventral axis reversed, they usually united with the host by all four cut surfaces. Within 12 hours after implantation the compounds were actively crawling. However, in contrast to the first described grafts, the anterior ends of these were continuously raised above the substrate as if the ventral longitudinal muscles of the graft were in a state of contraction.

In 5 cases the anterior tip of the host disintegrated within the first week after implantation. The presence of the graft prevented the regeneration of the host, and the implant itself reproduced the missing parts from its anterior surface (Fig. 27 and Pl. I, Fig. 28).

In all cases in which the dorsoventral axis was reversed, there was some proliferation of tissue between the graft and the host. In 4 cases both graft and host were involved, with the resulting development of one or two additional heads. The dorsal one was composed of the dorsal surface of the host and the ventral surface of the graft, and the ventral one, below the host head, consisted of the dorsal surface of the graft and ventral surface of the host. In 2 cases the ventral supernumerary head was bipolar.

In two cases the graft and host united by the dorsal surfaces only. In these the graft regenerated an anterior end, auricles, a functional pharynx, and a tail. During the 10 months that they were under observation the grafts, which showed greater muscular activity than their hosts, likewise showed relatively greater growth. By the end of the period, the two components were approximately the same size and crawled equally well whether the host ventral surface or that of the graft was in contact with the substrate (Pl. I, Fig. 29). After feeding them blood, it was possible to determine that the digestive tracts of graft and host were anastomosed, but behavior tests indicated that there were no extensive connections between the nervous systems of the components. A remarkable feature of these examples—one by which they differed from the others of this series—was that the grafts retained their specific characters during the entire period of observation. This condition will be treated in the discussion.

Dorsoventral anteroposterior orientation of graft (11 cases).—Only a few grafts were made with their dorsoventral and anteroposterior axes reversed. As was true in the other cases of inversion of the dorsoventral axis of the graft, there was a tendency for the anterior edge of the implant to fail to unite with the host. In only 3 instances were there complete takes. These resembled those of the series mentioned above in their behavior,

crawling with their heads continuously raised from the substrate. There was more or less proliferation of tissue between the graft and host, and in 1 case this was sufficient to inclose the graft completely. In this animal, eyes regenerated in the host and the graft regressed until it became a small projection on the anterior part of the host head. In the others the graft remained and no host eyes developed.

Incomplete takes usually failed to establish connections with the digestive tract of the host. Although they regenerated a more or less normal head, and in one instance a tail as well, they rapidly decreased in size and did not prevent the regeneration of a head by the host in cases in which it had previously disintegrated. In one incomplete take the digestive tract of the implant united with that of the host and the graft completely prevented any regeneration at the anterior end of the host (Pl. I, Fig. 30). In these, as well as in other dorsoventral grafts, there was no indication that the dorsal or ventral epithelia were modifiable.

Grafts in the anterior prepharyngeal region (20 cases).—Since the head contains the enlarged ganglionic part of the nervous system, there is the possibility that the resistance to induction at this level is a function of the amount of nervous tissue present. Accordingly, 20 grafts were implanted just behind the head. Not all possible combinations of dorsal and ventral with anterior and posterior surfaces were used, but the results were sufficient to show that there was no marked reduction in resistance in this level.

When grafts in normal orientation united completely with their hosts, there was no proliferation of tissue; and the only indication of induction was the appearance of a projection at each side of the implanted eyes (Pl. I, Fig. 31).

Figures 32 (Pl. I) and 33 show cases in which the grafts in normal orientation united by their posterior and a part of their lateral surfaces. A small postcephalic outgrowth developed in each case and subsequently tore through the right side of the host. The two differ in that in Figure 32 the graft and host heads are approximately equivalent, whereas in Figure 33 the host head has become reduced in size as a result of insufficient food reaching it from the branches of the digestive tract.

In Figure 34 we see a perfect take in which the anteroposterior axis was reversed. There was a slight proliferation of tissue around the graft, but otherwise no modification of the structure of the host was observed. Implanting the graft in reversed dorsoventral polarity usually resulted in union with the dorsal surface only. The grafts regenerated in nearly normal shape and induced a very small outgrowth of tissue (Figs. 35 and 36), producing a structure which resembled more or less several of Santos' (1931) figures (Figs. 4, 5, 6, 9, 13, and 14). There were two complete takes of dorsoventrally reversed grafts. In both of these a head developed composed of dorsal surface of the host and ventral surface of the graft (Fig. 37). In one the head developed from the anterior edge of the window and posterior edge of the graft, and in the other vice versa. In none of the grafts in this level was there any marked reduction of host resistance to reorganization. Accordingly, it may be concluded that the differential from anterior to posterior in resistance to induction is not dependent upon the presence or absence of certain structures concentrated in the most anterior levels, such as the cephalic ganglia.

GRAFTS IN THE PHARYNGEAL REGION (113 CASES)

When a cephalic graft was implanted into the pharyngeal region, the regenerating pharynx appeared some distance posterior to the original location, whereas that of the controls developed in the new tissue which marked its original position. Since the

pharynges of the experimental animals developed out of their normal locus, they may be designated as structures induced by the grafts. In contrast to those placed in the head, grafts in the pharynx induced the development of postcephalic outgrowths even in cases of complete union with the host. However, decapitation of the host at varying distances from the graft gave little evidence of graft dominance more than 2 mm. anterior to the level of implantation. Because of removal of the pharynges of the hosts, usually 3 or more weeks elapsed between the operation and the first feeding. During this time a tendency for the grafts to migrate was noted; and, accordingly, in certain instances longer periods of starvation were instituted, and cases of fusion of grafts with the host heads resulted. In both the pharynx and the tail regions the orientation of the implant had far less effect upon the final result than did the presence or absence of a

GRAFTS IN THE HEAD REGION

E. dorotocephala into *E. tigrina*.

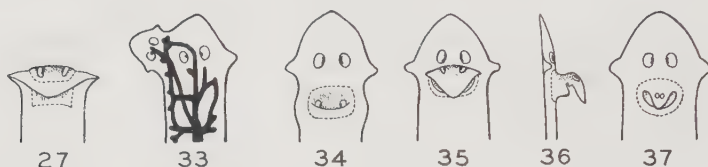


FIG. 27.—DVAA. Union complete. 7 days. New tissue around ventral surface of implant. Crawled with anterior portion of head raised. (Pl. I, FIG. 28.—Photograph of same animal 266 days later.)

GRAFTS IN THE ANTERIOR PREPHARYNGEAL REGION

FIG. 33.—DDAA. Anterior and right sides of graft failed to unite with host. 56 days. Graft larger than host head and controlled more posterior regions of host. Cephalic lobes, head shape, and epithelia of implant resembled those of host.

FIG. 34.—DDAP. Union complete. 21 days. New tissue partially covering anterior portion of implant. No appreciable change was observed during next 9 weeks.

FIGS. 35 (DORSAL VIEW) AND 36 (LATERAL VIEW).—DVAA. Union by posterior portion of dorsal surface only. 21 days. Short postcephalic outgrowth present. No appreciable change noted during next 9 weeks.

FIG. 37.—DVAA. Union complete. 21 days. Graft nearly inclosed by new tissue. Teratophthalmic outgrowth with dorsal epithelium of host and ventral epithelium of implant. No change noted during next 9 weeks.

free surface. For this reason the data in these sections will be described under two headings only.

Complete takes (61 cases).—Complete takes of cephalic grafts in the pharyngeal region generally induced tubular outgrowths which projected either from the dorsal or ventral surface of the host. (In 1 case no outgrowth developed, but in this it was noted from the first that the implant did not show the motor activity that was characteristic of all cephalic grafts.) When the eyes of the graft were subterminally located in the outgrowth, supernumerary photoreceptors developed at about the same level as those of the graft. This occurred even when the dorsal epithelium of the outgrowth was invaginated, as was the case when the outgrowth extended ventrally (Fig. 38). When the outgrowth developed at right angles to the anteroposterior axis of the graft, modification of the polarity of the graft was demonstrated by the development of eyes at right angles to

those of the implant and occasionally the complete resorption of the original eyes (Fig. 39). When the eyes of the implant were located terminally, they did not regress, and none were induced in the host (Fig. 40). These facts may be interpreted as follows. When there is no part of the outgrowth representing a more anterior level than that of the eye-level, the eyes of the graft take up a terminal position in the outgrowth. In this case the rest of the graft and the induced portions of the outgrowth which correspond to more posterior levels do not produce eyes. On the other hand, if the eyes were sub-terminal, the more anterior portion of the outgrowth produces, at a certain distance from the tip, the physiological level at which eyes can develop or persist. Since the outgrowth is tubular, the dominance extends radially, resulting in a ring of eyes which in these experiments varied from two to eight, depending upon the diameter of the outgrowth and the "scale of organization" (Child, 1931).

Figure 41 shows the only complete take in this region in which a pharynx developed anterior to the graft. This was located in reversed polarity and may be assumed to have been induced by the implant. It was considerably smaller than the one which appeared posterior to the graft. That there had been a complete reversal of polarity of this portion of the host was proved when there was no regeneration following the removal of the host head a short distance in front of the anterior pharynx.

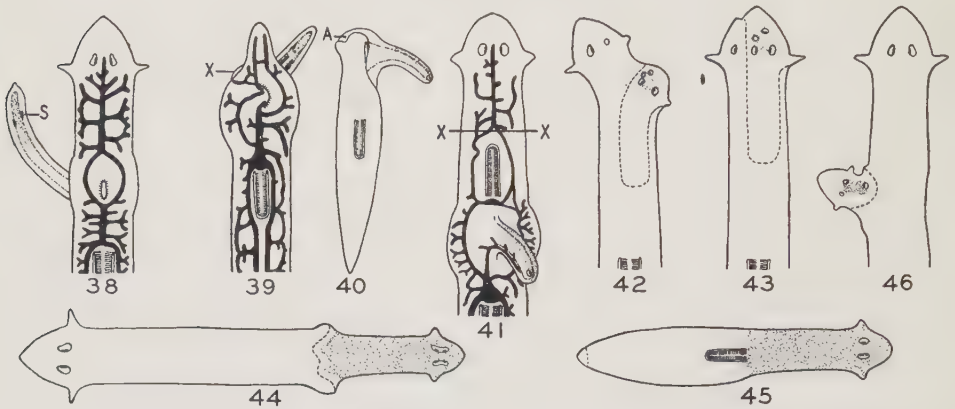
A gradual migration cephalad of grafts completely united with their hosts was noted in 9 cases. This was not as rapid as was observed in some cases of incomplete union, but in 1 case resulted eventually in fusion between the graft and host heads (Figs. 42 and 43). A rather significant feature of this migration was the disappearance of the cephalic lobe and eye of the host on the side nearest the graft. As the latter approached, these structures decreased in size and lost their pigmentation in a manner resembling stages in starvation. On the other hand, unpigmented new tissue was being laid down posterior to the graft, leaving a well-marked migration path behind. This indicated that host tissue was being used up anterior to the implant, while growth was taking place in the cells directly behind the graft. Consideration of these phenomena is reserved for the discussion.

In 8 cases grafts which united completely with the host were given a free surface. Four of these were given a free lateral and 4, a free posterior surface. In every case the anteroposterior axis of the resulting outgrowth was determined by the free surface and not by the original polarity of the graft. Figures 44 and 45 show a case in which a free posterior surface resulted in a 180° modification of graft polarity and the development of two additional eyes. These later fused with the original graft photoreceptors. Figure 46 shows a case in which a free lateral surface resulted in a modification of about 80° in the graft polarity.

In 3 cases the polarity was modified twice in the course of the observations. These were complete takes which were subsequently given a free lateral surface by a V-shaped cut in the side of the host. Figures 47 and 48 show the most clear-cut case. Freeing the lateral surface of the graft resulted in the development of a cephalic lobe at the anterior and another at the posterior margin of the graft and a photoreceptor beside the right eye of the graft (Fig. 47). These fused to form the right eye of the composite head. Meanwhile, as the graft migrated cephalad, the left side of the outgrowth fused with the right side of the host head. The right eye of the host and the two approximated cephalic lobes were resorbed (Fig. 48). Thus the polarity of the graft, after having been modified about 50° , finally, came to approximate its original condition.

Incomplete takes (52 cases).—The results of incomplete union differed from those of perfect takes in degree rather than in kind. In general the induced outgrowth of host tissue was greater and there was evidence of graft dominance extending a greater dis-

GRAFTS IN THE PHARYNGEAL REGION



FIGS. 38, 42, 43, 44, AND 45.—*E. tigrina* into *E. dorocephala*. FIGS. 39 AND 46.—*E. tigrina novangliae* into *E. dorocephala*. FIGS. 40 AND 41.—*E. dorocephala* into *E. tigrina novangliae*. Note that in all cases the reconstituted pharynx is posterior to original pharynx level (level of implantation).

FIG. 38.—DDAA. Union complete. 42 days. Tubular outgrowth developed covered with ventral epithelium and containing two supernumerary eyes (*S*, only one shown).

FIG. 39.—DDAP. Union complete. 35 days. Original eyes disappeared, and four pigmented areas developed. Tail-like outgrowth developed from right side of host anterior to implant. Host, decapitated at *X* (about 2 mm. from implant) 1 week earlier, completely failed to regenerate.

FIG. 40.—DDAP. Union complete. 42 days. Large graft (1.4 × 1.1 mm.). Ventral view. Large tubular outgrowth has developed with terminally located eyes. Decapitated 4 weeks earlier about 3 mm. from graft. Regeneration limited to small triangle of tissue (*A*).

FIG. 41.—DDAP. Union complete. 28 days. Large graft (1.3 × 1.0 mm.). Pharynx with reversed polarity induced anterior to graft. Extent of graft dominance cephalad indicated by anterior reorganization of digestive tract. Host failed to regenerate after section at *X-X* 14 days later.

FIGS. 42 AND 43.—DDAA. Implanted to right of middle of host. Union complete. FIG. 42.—63 days. Path of graft migration indicated by new tissue. Nearest cephalic lobe and eye of host reduced in size with pigmented area entirely absent. Unpigmented outgrowth anterior to left graft eye. FIG. 43.—77 days. Entire right side of host head replaced by graft and surrounding tissues.

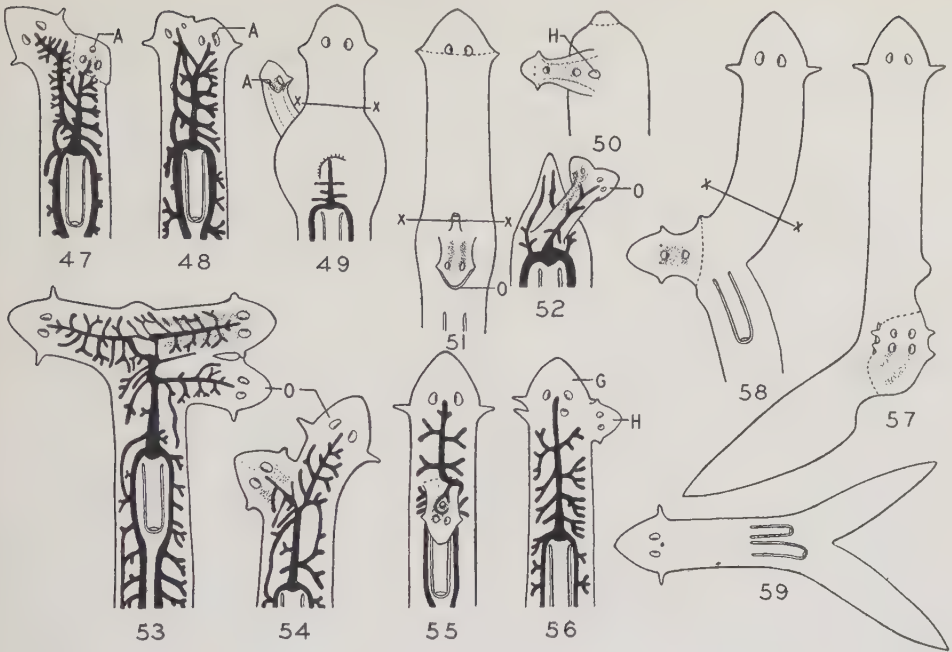
FIGS. 44 AND 45.—DDAA. Large graft (1.3 × 0.9 mm.). Union complete, but graft given a free posterior surface 7 days after implantation. FIG. 44.—31 days. Graft polarity modified 180°, and two pigmented areas developing behind original ones. Line between graft and host still distinct. FIG. 45.—74 days. Graft retained specific characters and reversed the polarity of a portion of host, as indicated by ciliary movement, orientation of pharynx, and regeneration of a tail after fission.

FIG. 46.—DDAA. Union nearly complete. 28 days. Left side of graft cut free from host 18 days after implantation. Left graft eye became right eye of outgrowth, and right graft eye eventually disappeared. Six weeks later graft had migrated cephalad, producing a Y-shaped compound resembling Figs. 54 and 61.

tance cephalad than in the case of complete takes. Of the four surfaces of the graft, the posterior united with the host most frequently (44 cases, 85 per cent).

In 13 cases the graft united by three of its four cut surfaces, and tubular outgrowths open at both ends resulted. In 4 of the foregoing the free surface was lateral. In 3 of

GRAFTS IN THE PHARYNGEAL REGION



FIGS. 47, 48, 50-59.—*E. tigrina novangliae* into *E. dorotocephala*. FIG. 49.—*E. dorotocephala* into *E. tigrina novangliae*. FIGS. 49, 50, 57-59.—DDAA. FIGS. 51-56.—DDAP.

FIGS. 47 AND 48.—42 and 63 days, respectively. Union complete. FIG. 47.—V-shaped incision made in right side of host opposite graft 1 week earlier. Eye (A) has developed distal to left graft eye. Cephalic lobes developing anterior and posterior to implant. Modification of graft polarity about 50° . FIG. 48.—Graft and host heads fusing. Right eye and auricle of host regressing. Unpigmented area of supernumerary eye (A) fused with right graft eye. With resorption of right side of host head, fusion was complete and polarity of graft returned to approximately original condition.

FIG. 49.—Right anterior corner of graft failed to unite with host. 28 days. Tubular outgrowth covered with ventral epithelium. Eye (A) developed opposite left graft eye. Graft polarity modified about 40° . Host decapitated at x-x regenerated normal head in 2 weeks.

FIG. 50.—Part of left and right sides of graft failed to unite with host. 21 days. Small head with two pigment spots has developed from left side of graft. Eye beside hole (H) on right side did not regress during 7 weeks of observation. Regeneration of host inhibited.

FIGS. 51 AND 52.—14 and 35 days, respectively. Anterior surface of graft failed to unite with host. FIG. 51.—Small pharynx with reversed polarity induced anterior to graft. Head has developed from host portion of tubular outgrowth (O). Sectioned at x-x, tail with two-branched digestive tract developed (Fig. 52).

FIGS. 53 AND 54.—35 and 96 days, respectively. Anterior and part of lateral surfaces of graft failed to unite with host. Large graft (1.5 X 1.1 mm.). FIG. 53.—Head (O) regenerated from free host surface behind graft. Right side of host tore apart at level of implant. FIG. 54.—Host head removed 3 weeks earlier failed to regenerate. During following 8 weeks host epithelia completely replaced those of graft.

FIGS. 55 AND 56.—35 and 105 days, respectively. Right anterior edge of graft failed to unite with host. FIG. 55.—Graft polarity modified about 70° , and third eye has developed. FIG. 56.—Graft epithelia replaced, but implant (G) has migrated cephalad. Greatly reduced host head (H) disintegrated 1 week later.

FIG. 57.—Sides of graft failed to unite with host. 31 days. Portion of host lateral to implant tore apart. New tissue anterior to graft has developed eyes and auricles with reversed polarity.

FIGS. 58 AND 59.—21 and 49 days, respectively. Left side of graft failed to unite with host. FIG. 58.—Host lateral to graft tore apart 1 week earlier. Modification of graft polarity indicated by position of auricles and of two pigmented areas. Following decapitation at x-x, tail and second pharynx developed in host anterior to graft. FIG. 59.—Graft eyes reduced to pigmented areas only.

these cases a modification in polarity was indicated by the position of the regenerated eyes and cephalic lobes. In the fourth, in addition to a large part of the left side of the graft failing to unite, there was a small hole between the graft and host on the right (Fig. 50). The right graft eye close to the hole did not regress, as generally occurred when there was a modification of 90° in the graft polarity (compare with Figs. 46, 58, and 59).

The 5 cases in which the anterior surface of the graft was free developed larger outgrowths than did the 3 cases mentioned above. In 3 of the 5 cases the outgrowths were ventral (Fig. 49); in 2, dorsal. In 2 cases there was a slight modification of polarity, and in 3 the free surface was approximately parallel with the eyes and polarity remained unchanged.

In 4 cases with reversed anteroposterior axis, in addition to the anterior surface of the graft, a small portion of each lateral surface failed to unite. In each case two cephalic lobes were regenerated and a tubular postcephalic outgrowth was induced. From the free surface at the posterior edge of the host window a secondary head developed. In 1 case (Figs. 51 and 52) a very small but functional pharynx developed in reverse orientation just anterior to the transplant. Following decapitation, a tail with the characteristic two-branched digestive tract developed from the anterior cut surface. In the second case the graft and secondary head tore through the right side of the host and thus obtained access to the substrate. During the next 3 weeks they were almost constantly pulling against the host head, taking on the appearance of the letter *T* (Figs. 53 and 54). Great difficulty was experienced in inducing this specimen to feed. During 4 weeks of starvation subsequent to the condition shown in Figure 53, the original host head was reduced to a size about equal to that of the graft head. In an attempt to prevent the excessive stimulation of three heads in the presence of food, the original head of the host was removed at this time. The cut surface healed, and the two remaining heads took up an anterior position. However, it was not until 3 weeks later that, as a result of being placed in the dark at feeding time (cf. above, p. 218), the specimen began taking food again. At this time it was only 1.6 mm. long, and the secondary host head was larger than that of the graft (Fig. 54). As it increased in size, the graft head grew more rapidly, until it again became equivalent to that of the host. During the 7 weeks of complete starvation the line of demarcation between graft and host remained quite distinct. However, as the specimen increased in size, the graft epidermis was observed to fade into host tissue along the line of separation until eventually the photoreceptors, head shape, and cephalic lobes resembled those of the host. This situation will be treated in the discussion.

In the third case, only a small portion of the right side of the graft was free, while a considerable portion of the left did not unite with the host. During regeneration of the graft a third eye appeared anterior to the left eye, and cephalic lobes developed opposite it and the right eye of the graft (Figs. 55 and 56). The implant increased in size as it migrated cephalad, and eventually dominated the host, while the host head became merely an appendage (Fig. 56). The fourth resembled the third except that more of the right side of the graft was free and there was no alteration of graft polarity. As a result of failure of the digestive tract of the graft to unite with a main branch of that of the host, the graft and the secondary host head received insufficient food and eventually regressed.

Two of the implants united by their anterior and posterior surfaces, leaving the larger portion of the lateral surfaces free. In both cases the eyes developed in the portion of

the host in contact with the anterior part of the graft, i.e., in reversed orientation. In one, two structures resembling cephalic lobes developed in host tissue lateral to the induced eyes (Fig. 57). These appear to be another expression of the same situation observed in the development of photoreceptors in the tubular outgrowths resulting from complete takes. It is evident that the distance from the anterior edge of the graft to the graft eyes in one direction and to the induced eyes in the other was approximately equal. If the two lateral surfaces of the graft and host were fused, a situation resembling that in Figure 77 would result.

There was only 1 case in which a graft united with the host by one of the lateral surfaces. Two weeks after implantation it tore through the tissue on the left side of the host. The next week two pigment spots appeared distal to the left one of the graft, and a pharynx developed posterior to the level of implantation (Fig. 58). At this time the host head was removed. Instead of a head, a tail developed from the cut surface, and later a second pharynx appeared in reversed polarity (Fig. 59).

In 30 cases only the posterior surface of the graft united with the host. Figures 60 and 61 represent the typical result in this group. By an increase in size of the graft and a decrease in size of the host prepharyngeal region a Y-shaped compound resulted.

Five hosts tore apart either anterior or posterior to the implant. When the graft remained attached to the posterior piece, there was no regeneration of the host (Fig. 63). In these the graft formed the anterior portion of the worm. When the graft was attached to the anterior half, one or two tails developed from the exposed surfaces of the host. However, no pharynges developed, and the specimens gradually decreased in size.

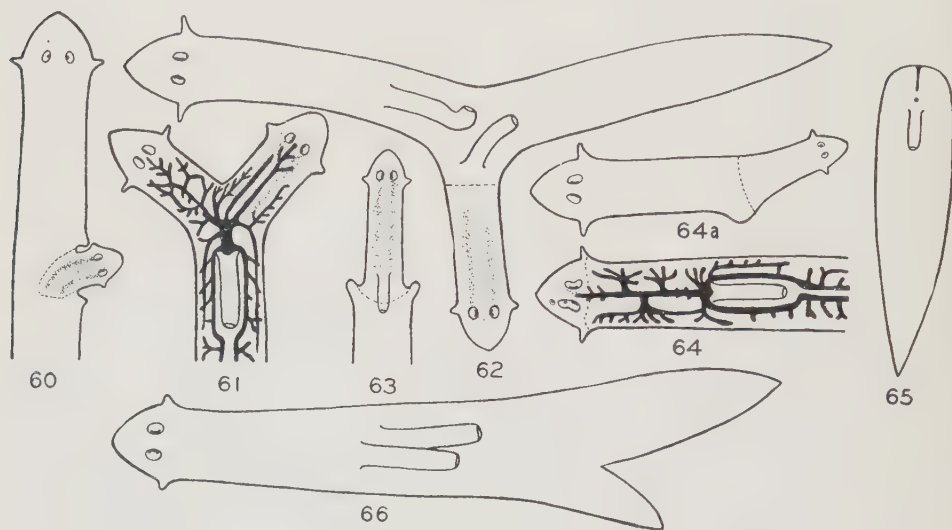
In 5 cases the windows were made by removing the dorsal wall and the underlying mesodermal tissues without cutting through the ventral wall. Grafts placed in these united with the dorsal surface of the host only. In addition there were 3 cases in which, although the usual procedure was followed, the ventral surface was not involved in the union. All 8 examples regenerated the triangular head shape and cephalic lobes but within 2 or 3 weeks became greatly reduced in size and showed evidences of lack of nourishment. When fed blood, it could be seen that no connection had been established between the digestive systems of graft and host.

Grafts placed in reversed dorsoventral orientation usually united with the host by their posterior surfaces, with the anterior tip of the graft bending back upon itself. When union of digestive tracts of graft and host occurred, the grafts persisted; otherwise they decreased in size and were lost.

Removal of the host head.—In 45 cases the host was decapitated 2 weeks or more after transplantation. Thirty were cut close to the graft (i.e., about 1–2 mm. anterior to it). In 18 of these regeneration of another host head was completely prevented (Figs. 39, 40, 50, 52). In the other 12 cases a normal head regenerated, but in 5 of these the grafts were regressing at the time of decapitation and so might perhaps be excluded from consideration. In 7 cases the section was made about 3 or 4 mm. from the graft. Five of these regenerated normal heads, and in only 2 cases was host regeneration inhibited (Fig. 59). In 8 cases the host was decapitated a short distance posterior to the host head. The only specimen that did not regenerate a normal head was one mentioned earlier (Fig. 41) in which the graft had induced a pharynx in reversed polarity anterior to the level of implantation. All the controls regenerated normal heads. It is evident that dominance of cephalic grafts does not usually extend much more than 2 mm. cephalad in large planarians such as were used in these experiments.

In 3 cases removal of the host body anterior to the graft, by section as near as possible to the graft, resulted in development of bipolars with a small but normal head instead of a tail at the posterior surface of the piece removed (Figs. 64, 64a). In these the polarity reversal was so firmly established that removal of the dominant region under which it had developed did not destroy it.

GRAFTS IN THE PHARYNGEAL REGION



FIGS. 60-64.—*E. tigrina novangliae* into *E. dorotocephala*. DDAA. FIG. 65.—*E. dorotocephala*, control. FIG. 66.—*E. tigrina novangliae*, control.

FIGS. 60 AND 61.—Anterior surface of implant failed to unite with host and right side of host tore apart at level of implantation. FIG. 60.—14 days. Graft has regenerated cephalic lobes. FIG. 61.—42 days. Graft has increased in relative size and induced a pharynx. During next 9 weeks the two heads remained approximately equivalent.

FIG. 62.—Graft united to host by posterior surface. 35 days. The only case in which a pharynx with normal polarity appeared anterior to level of implantation.

FIG. 63.—Graft united by posterior surface. 14 days. Host tore apart anterior to implant 1 week earlier. No regeneration from exposed surfaces of host.

FIGS. 64 AND 64a.—Union complete. 35 days. 21 days after subsequent section of host just cephalad to implantation level. FIG. 64a.—Anterior part of host has regenerated a head on posterior surface. FIG. 64.—Posterior part of host containing implant has developed cephalic lobes. Two supernumerary eyes have appeared anterior to left graft eye.

FIG. 65.—Control. 7 days. Lateral margins of window united and animal tore apart. Posterior portion of animal developed single pigment spot and small pharynx but refused food.

FIG. 66.—Control. 63 days. Window in host remained open for more than a week. Incision made in right side 4 weeks after operation. Three weeks later tail-like projection appeared. Second pharynx appeared 2 weeks later.

Controls.—With only a few exceptions the tissue which was removed was replaced in the controls and a functional pharynx regenerated. In 1 case the window did not fill in completely, and a small head regenerated from its posterior edge. In another the two

sides fused together, and, when the specimen tore apart subsequently, there was no regeneration at the anterior surface. A single median eye and a pharynx developed, but the piece would not feed (Fig. 65). In several controls in which there was little tissue along the sides of the window, the worms tore apart at this point. Later a head regenerated on the posterior piece and a tail on the anterior. In 3 cases after healing, tail-like outgrowths developed behind the level of operation. Figure 66 shows a case in which a pharynx developed in association with such an outgrowth.

GRAFTS IN THE TAIL (174 CASES)

The tail proved to be the most labile of the regions studied. Cephalic grafts placed in this region induced the greatest outgrowths and produced the greatest reorganization of the host. Pharynges were induced equally well anterior or posterior to the level of implantation. They developed about the same distance anterior or posterior to the graft, but their lengths and diameters were proportional to the amount of tissue in which they developed. There were a few cases in which, in addition to a pharynx, a tail was induced anterior to the graft. Data on the range of dominance anteriorly showed that, unless the host was unusually long, the inhibitory power of the graft extended about to the fission zone of the host and occasionally into the pharyngeal level itself.

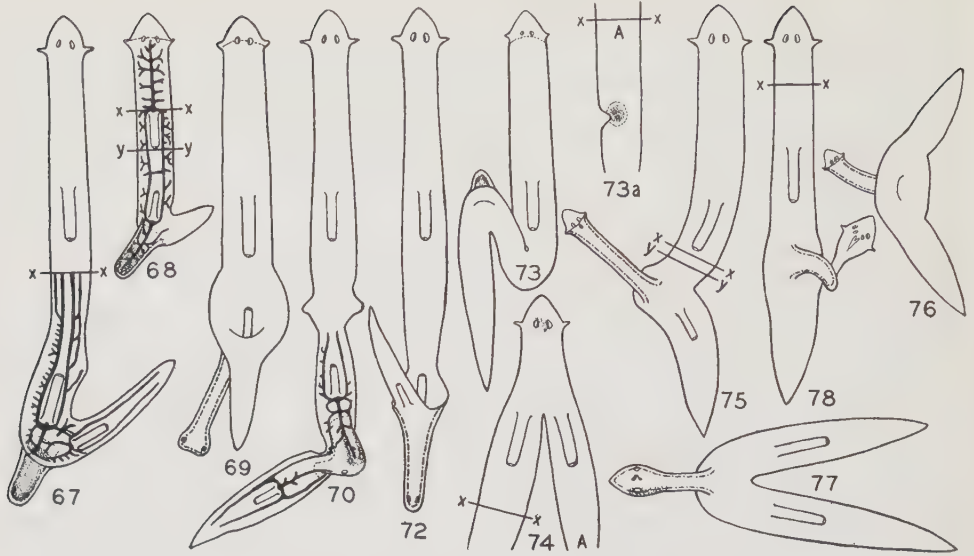
When the two-tailed forms produced by removal of the host heads were followed for long periods (6 months to a year), they were observed to fuse along the adjoining lateral margins, eventually producing double monsters. The fission products of these forms usually developed three or four eyes, two pharynges, and a more or less completely doubled digestive tract.

A few triangular implants containing chiefly ganglionic material were placed in the tail. Although containing less tissue than the rectangular grafts used generally in this study, they showed greater inductive capacities than the latter. No tendency to migrate was observed in implants in this level.

Complete takes (71 cases).—Cephalic grafts in the tail which united completely with their hosts produced the same type of tubular outgrowths as did complete takes in the pharynx. When the photoreceptors came to lie in the terminal portion of the outgrowth (43 cases), there was no alteration of graft polarity (Figs. 67, 68, and 69). When sub-terminal, supernumerary eyes developed in the induced tissue (Fig. 70). These outgrowths generally were larger than those resulting from grafts in the pharynx level (Pl. I, Fig. 71). Also, it usually was possible to observe that control by the graft extended some distance anterior to the level of implantation.

Pharynges were induced in the host in 19 cases. Of these, 13 developed two supernumerary pharynges, one posterior to the graft in normal orientation and the other anterior to it with reversed anteroposterior axis (Figs. 67, 70, and 72). In 4 of the 6 cases in which there was but one induced pharynx, it was posterior to the graft, while in the other 2 it was anterior (Fig. 69). In the latter the absence of a posterior pharynx may have been the result either of the small amount of host tissue posterior to the graft or of the proximity of the posterior tip of the tail, or both. In this connection it should be mentioned that there appeared to be a relationship between the length and diameter of the pharynx and the amount of tissue in which it developed, but the level at which it appeared was determined by the implant. Figures 67, 70, and 72 illustrate this situation. In these, both induced pharynges developed at approximately the same distance from the graft; but the posterior one, extending into the narrow tip of the tail, was both

GRAFTS IN THE TAIL REGION



FIGS. 67, 68, 70, 72, 75, 76, AND 78.—*E. tigrina novangliae* into *E. dorotocephala*. FIG. 69.—*E. dorotocephala* into *E. tigrina novangliae*. FIGS. 73 AND 74.—*E. tigrina* into *E. dorotocephala*.

FIGS. 67 AND 68.—DDAP. Union complete. 21 days. Host fissioned anterior to implant and normal head and pharynx reconstituted.

FIG. 67.—Digestive tract reorganized and pharynx developed anterior, as well as posterior, to implant. Note that reversed pharynx is larger (also in Figs. 70 and 72). Following section at *x-x*, tail regenerated on posterior piece.

FIG. 68.—Digestive tract reorganized, and pharynx developed anterior to implant. Digestive tract was not seen in tip of tail, since no food entered. Normal head regenerated following decapitation at *x-x*. Tail developed following section at *y-y* 3 weeks later.

FIG. 69.—DDAA. Union complete. Graft has induced tubular outgrowth and pharynx with reversed polarity.

FIG. 70.—DDAP. Union complete. 14 days. Digestive tract reorganized and pharynxes developed anterior and posterior to implant. Two eyes (only one figured) in new tissue posterior to graft.

FIG. 72.—Implants placed in both head and tail. DDAA. Union complete. 35 days. Graft in head has been incorporated into host. Pharynxes have developed anterior and posterior to ventrally projecting tubular outgrowth induced by second graft.

FIGS. 73 AND 74.—Union complete. DDAA. 14 and 35 days, respectively. FIG. 73.—Ventrally projecting tubular outgrowth has developed and posterior part of host is under graft control. FIG. 73a.—Portion of animal after V-shaped incision was made, giving graft a free surface. One week later host fissioned at *x-x*. FIG. 74.—Original eyes reduced to pigmented spots. New eyes, cephalic lobes, and two pharynxes have developed. Tail, instead of head, has regenerated at *A*. One week later animal fissioned at *x-x*.

FIG. 75.—DDAA. Left and posterior edges of graft failed to unite with host. 28 days. Graft polarity modified about 120°, and new eye has developed. Digestive tract (not shown) has reorganized. Tubular outgrowth and pharynx have developed posterior to implant. No regeneration occurred when sectioned at *x-x* 4 weeks later. After second removal (*y-y*), 8 weeks later, tail regenerated and second pharynx with reversed polarity developed anterior to implant.

FIG. 76.—DDAA. Anterior surface of implant failed to unite with host. 21 days. Tail, instead of head, regenerated on host following fission 1 week after implantation. Graft has induced a tubular outgrowth lined with dorsal epithelium.

FIG. 77.—DDAA. Very small part of right surface of graft failed to unite with host. 35 days. Implant induced tubular outgrowth with two eyes (one not shown) opposite those of graft and two pharynxes. From hole two auricle-like structures developed. Anophthalmic head developed after fission of host 5 weeks earlier. When removed after 2 weeks, a taillike structure regenerated.

FIG. 78.—Triangular graft DDAA. Right side of graft and very small part of left failed to unite with host, leaving a hole on left. 56 days. Tubular outgrowth developed posterior to graft. Second pair of eyes developed between hole and lateral surface of outgrowth. Host regenerated normal head after section at *x-x*.

shorter and smaller than the anterior. Some of Santos' figures (Figs. 26, 29, 36, and 37) show the same condition, but he does not comment on the situation.

In 5 cases the host tissue on one side of the graft was removed, thus giving the graft a free surface. All 5 regenerated cephalic lobes and one or two eyes; and in each case the final polarity of the outgrowth was determined by the free surface (Figs. 73, 73*a*, and 74). There was no indication in these of migration such as was described for grafts in the pharynx.

Incomplete takes (103 cases).—Grafts in the tail which did not unite completely with their hosts regenerated their missing parts more or less completely, depending upon the amount of free surface. When union was nearly complete, tubular outgrowths developed; but when one or more sides of the graft remained free, the resulting outgrowth was flat. As was true for other levels, the polarity of the outgrowth was determined by the free surface. This was shown by the cases in which it did not coincide with the original anterior surface of the graft (Fig. 75).

In 16 cases union was nearly complete, and a tubular outgrowth developed. In 14 of these the outgrowth extended dorsally with the dorsal surface of the graft and host outside (Fig. 75). In the other 2 cases it was ventral with the dorsal surface inside (Fig. 76).

In 5 cases a very small portion of one edge of the graft did not unite, resulting in a hole between the graft and host. One or two cephalic lobes regenerated from this; and in the cases in which union was otherwise complete, a pair of eyes developed in the host portion of the outgrowth at the same level as those of the implant (Fig. 77). In 3 instances in which the graft united by only one surface, a second pair of photoreceptors developed between the hole and the lateral surface of the outgrowth, resulting in a compound head with three cephalic lobes and four photoreceptors (Figs. 78 and 79). These cases resemble rather closely that shown in Figure 57, in which a pair of eyes and two cephalic lobes developed in the new tissue anterior to an implant in the pharyngeal region. In 1 case a graft eye close to such a hole persisted for 14 weeks in a distinctly ectopic position, whereas usually photoreceptors at such a distance from the normal locus disappeared completely in 4–6 weeks (Fig. 82).

When the grafts in this level united by one edge, it was commonly the posterior (36 cases). In these there was the usual regeneration of missing parts by the graft head and reorganization of the host both anterior and posterior to the level of implantation. Since cases similar to those obtained in this study have been described and figured by Santos (1931), they will not be taken up in any detail. In 4 cases a lateral surface of the graft united with the host. In these, as with some of the foregoing, in which a portion of a lateral, as well as the posterior, surface was involved in the union, there was the usual modification in the polarity of the implant.

In a number of cases there were indications of the development of a tail between the host pharynx and the graft-induced one. These appeared first as slight swellings, which, as they grew, became supplied with a two-branched digestive tract. There were varying degrees of development of these outgrowths from slight projections to well developed tails. Figure 83 (Pl. I) is a photograph of one in which the supernumerary tail was well developed.

As was the case in other levels, reversal of the dorsoventral axis resulted in a proliferation of tissue at the line of union between graft and host. There was a tendency for the union to be superficial, involving only the two dorsal surfaces (Pl. I, Fig. 84). This was probably a result of the greater contraction of the ventral than the dorsal sur-

face of the grafts when they were inverted. Incomplete unions were very common in these grafts, and the host window often tore apart directly anterior or posterior to the graft. In Figure 85 (Pl. I) we see a case in which the two portions of host tissue lateral to the graft developed into tails after the original host tail had torn off. However, when both dorsal and ventral surfaces united, there often was regeneration of one or two heads and tails. In one of these the dorsal surface of the host was united with the ventral surface of the graft, and in the other the ventral surface of the host with the dorsal surface of the graft. Figure 86 (Pl. I) shows an interesting example of this sort. In this case the host underwent fission directly behind the implant. A host head developed with reversed polarity, apparently induced by the graft, and with its ventral surface continuous with that of the graft. A single functional pharynx was induced, probably by the combined dominance of the two heads.

Cephalic grafts in the tail with the anteroposterior axis of the graft reversed retained their polarity in cases of complete union and reorganized the host equally well as did grafts in normal orientation. With reversed anteroposterior orientation, however, the graft formed the anterior, rather than the posterior, half of the outgrowth; and the induced eyes were posterior, rather than anterior, to the graft (Figs. 68 and 70).

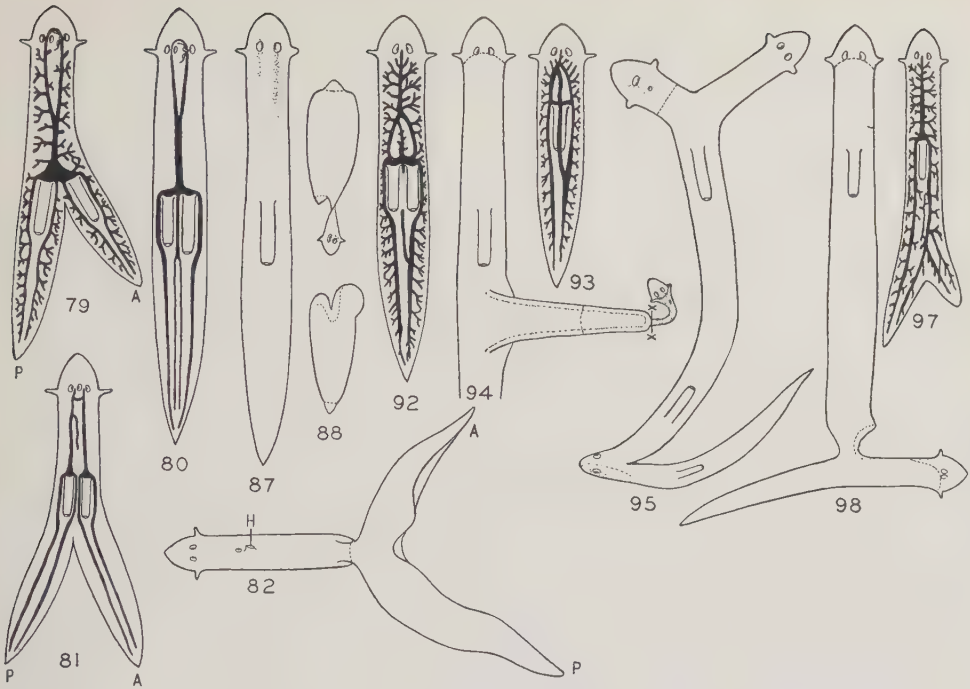
In cases of incomplete union, cephalic grafts in the tail with anteroposterior axis reversed gave results indistinguishable from those whose anteroposterior axes coincided with those of the hosts.

In 17 cases the host tore apart immediately anterior to the graft. In 14 of these the graft completely prevented regeneration from the exposed surfaces of the host. In the other 3 a tail, instead of a head, developed in the host. It was in grafts such as these that the persistence of the graft species characters was most marked. Figure 87 shows a case, 20 weeks after grafting, in which the species differences still were distinctly recognizable. The line between graft and host had faded out, but it was evident that the anterior and right portions of the compound were *E. tigrina* tissue while the posterior and left were *E. dorotocephala*. The persistence and replacement of graft tissues will be treated under "Discussion."

In 6 cases the posterior part of the host window tore apart, leaving the graft attached to the anterior piece of the worm. From the torn edges two tails developed in 1 case (Pl. I, Fig. 85), one tail in 3 cases (Pl. I, Figs. 89 and 90), and there was no regeneration in 2 cases (Fig. 88).

In 5 cases a pharynx was induced with reversed polarity. These grafts may be contrasted with similar ones in the pharyngeal region in which induction of a pharynx did not occur. In the latter the distance from the graft to host head was so short that there was no level of the gradient between them at which a pharynx could develop. However, in the case of implants in the tail the tissue between the two heads included the pharynx level, and reorganization by the graft expressed itself in the induction of a pharynx with reversed polarity.

In 40 cases the host head and part of the host body were removed 2 or more weeks subsequent to grafting. Of these, 10 were cut a short distance behind the host head, 10 about midway between the host head and the pharynx, 10 across the middle of the host pharynx, and 10 posterior to the pharynx. All of those cut close to the host head, and those cut in the prepharyngeal region regenerated normal heads anteriorly. Of those cut through the middle of the pharyngeal region, 4 regenerated normal heads and 6 developed tails, while all of those cut between the host pharynx and the graft regenerated



FIGS. 79-81, 92, AND 93.—*E. tigrina* into *E. dorocephala*. FIGS. 82, 87, 88, AND 94.—*E. tigrina novangliae* into *E. dorocephala*. FIG. 95.—*E. dorocephala* into *E. tigrina novangliae*. FIGS. 79-82, 87, 92-95.—DDAA. FIG. 88.—DVAA.

FIGS. 79-81.—133 and 216 days, respectively. Left and a part of right side of graft failed to unite with host. FIG. 79.—Short outgrowth of tissue with two auricle-like structures developed from hole on right of graft. Tail, instead of head, regenerated from anterior surface (A) following fission. Fusion between two tails (A and P) beginning. FIG. 80.—Only main branches of digestive tract are figured. Lateral fusion of tails has resulted in doubled postpharyngeal region. FIG. 81.—Fission piece of Fig. 80. Note presence of three eyes and completely doubled digestive tract.

FIG. 82.—Triangular graft. 35 days. Right and very small part of left side of graft failed to unite with host. Left graft eye, close to hole (H), remained 14 weeks before final disappearance. Replacement of graft epithelia almost complete. Tail has regenerated from anterior end of host (A) following fission.

FIG. 87.—Union by posterior surface only. Host tore apart at implantation level 1 week after operation. 119 days. Graft has retained its specific characters, although line of union is no longer distinct.

FIG. 88.—Triangular graft. Posterior surface of graft failed to unite with host. 14 days. Graft polarity modified about 140° . Host fissioned anterior to implant and tore apart posterior to it. Graft inhibited anterior regeneration and dominated behavior of piece but was lost 2 weeks later.

FIGS. 92 AND 93.—Anterior surface of graft failed to unite with host. 182 days. FIG. 92.—Regenerated posterior third of fission piece with doubled digestive tract. Note two pharynxes and partially doubled digestive tract. FIG. 93.—Piece fissioned from Fig. 92. Note single pharynx.

FIG. 94.—Triangular graft. Union by posterior surface of implant to dorsal surface only of host. 21 days. Original eye reduced to pigment spot, and two new ones have developed. Note long tubular outgrowth from host and small size of graft. Graft gradually decreased in size and eventually pinched off at X-X 6 weeks later. Induced outgrowth remained until termination of experiment on 105th day.

FIG. 95.—Double graft. Anterior implant in pharyngeal region, posterior in tail. Anterior united by right side only, posterior united completely. 21 days. Note three regions of influence in host: anterior under control of host head, middle under control of first graft, and tail dominated by second. Two weeks later, left eye of first graft was double, indicating 90° modification of polarity.

FIG. 97.—Control. *E. dorocephala*. 42 days. Fission occurred at level of window; and, following, regeneration of normal head, outgrowth containing two-branched digestive tract appeared.

FIG. 98.—*E. dorocephala*. Control for Fig. 73. 21 days. Right side of host window tore apart, and teratophthalmic head regenerated.

tails. In addition to this experiment, there were 58 cases of fission posterior to the pharynx but anterior to the implant. Of these, 33 developed tails anteriorly, 20 showed no outgrowth of tissue at all, and 17 regenerated heads. Some of the fissions here recorded occurred during the first week after operation. Of these, 10 developed normal heads, leaving only 1 case in which a normal head developed after the reorganization effects had begun to appear.

In all cases in which tails were induced, there was a tendency for the two tails to become parallel and fuse (Pl. I, Fig. 91). In those which were observed for long periods of time (up to 15 months) the two tails gradually approximated, until finally a worm with doubled pharyngeal and postpharyngeal regions resulted. When fission of this double animal occurred, the fission pieces usually developed two to four photoreceptors and a more or less completely doubled digestive tract, consisting, in the most extreme cases, of two anterior branches, two pharynges, and four posterior branches (Figs. 80 and 81). In 1 instance sixteen successive fission pieces from a single graft showed this condition. Sonneborn (1930) produced doubled forms of *Stenostomum* as a result of treatment with lead acetate. These abnormalities were passed from one generation to another by fission, and therefore he considers them to be hereditary modifications. From the same point of view, the modifications described above could be considered hereditary, since they depended upon a self-perpetuating pattern persisting from parent to offspring. However, it appears to the writer that a most important feature of this "inheritance" has not been taken into account. This is the fact that all the "offspring" resulting from fission are, in reality, parts of the parent and must necessarily partake of such axial modifications of the parent as the doubling described by Sonneborn. In the cases reported here, it is extremely improbable that there was any modification of the hereditary units which might persist through sexual reproduction. An experiment was performed with the planarians which, because of the very small number of animals, was inconclusive except in one respect. Two large double specimens of *E. dorocephala* were cut transversely into three approximately equal pieces and allowed to regenerate. The two anterior pieces regenerated a tail and a single pharynx. The two middle pieces, each containing two pharynges, regenerated a normal head and a tail containing a three-branched digestive tract. The posterior pieces regenerated normal heads and partially doubled digestive tracts, containing two pharynges and three posterior branches (Fig. 92). During the second week of regeneration fission occurred in the posterior pieces, and the fission pieces both reconstituted a single pharynx and almost normal posterior intestinal branches (Fig. 93). Further experiments along this line are necessary before the factors involved can be determined. However, since the same animal could produce single as well as double individuals, the persistence of the latter condition could hardly be called hereditary, even in the sense of Sonneborn.

Triangular grafts (33 cases).—The first grafts which were made in this study were triangular grafts in the tail region, made according to the method of Santos (1931). These implants contained less tissue than the rectangular grafts which were used later, but contained more of the cephalic ganglia. The postcephalic outgrowths induced by these were generally larger than those of the rectangular grafts, but often the host digestive tract did not unite with those of the grafts, and the latter did not persist (Fig. 94).

In only 3 of the 33 cases of triangular grafts did the ventral surface of the graft unite with that of the host; in the other 30 cases it united with the dorsal surface of the host and the host ventral surface healed over. As a result of this, tubular outgrowths which

ended blindly were induced even in cases of incomplete union (Figs. 78 and 94), and the frequency of union between graft and host digestive tract was very much less than in rectangular implants. Among the rectangular grafts there were only 3 cases in which the ventral surface of the host did not unite with some part of the graft.

Double grafts (12 cases).—The effect of the level in which a graft is placed was strikingly illustrated in a series of 12 double grafts. Figure 72 shows the results of simultaneous implantation of cephalic grafts in the head and in the tail of the host. Both were complete takes; but the graft in the head healed without leaving any traces, while that in the tail induced a tubular postcephalic outgrowth and two pharynges.

Figure 95 illustrates the result of simultaneous transplantation in the pharynx and tail. In this case the anterior graft united by the right side only, while the posterior united by all surfaces. Three well-marked regions of influence can be seen. That controlled by the host head extended about to the level of implantation of the first graft. The first graft controlled the movements of the host a short distance anterior to it and posteriorly about half the distance to the pharynx induced by the second graft. The second graft controlled about equal distances anteriorly and posteriorly. Figure 96 (Pl. I) is a photograph of a case in which grafts placed in the pharynx and tail both united with the host by all four surfaces. The three regions of influence evident in Figure 95 can also be seen in this illustration.

Controls.—The large majority of the controls replaced the parts removed, and within 2-4 weeks were normal again. The exceptions consisted chiefly of cases in which the lateral edges of the window united, effectively preventing food from reaching the tip of the tail, which eventually either tore apart or disintegrated. In two cases a tail-like outgrowth developed behind the level of operation, and one of these was observed to be supplied by two branches of the digestive tract (Fig. 97). In one case one side of the window tore apart, and from it a teratophthalmic head regenerated. This dominated the behavior of the more posterior part of the specimen and completely reorganized it (Fig. 98).

DISCUSSION

TRIANGULAR VERSUS RECTANGULAR GRAFTS

The increased frequency of takes by using rectangular, instead of triangular, grafts was mentioned in the section on "Methods." In addition to this, the use of rectangular grafts has clarified the problem of polarity of the implants. In cases where implant and host united completely, Santos (1931) shows figures of small monophthalmic outgrowths of undetermined polarity and states that they were the usual result. In the experiments here, such outgrowths were common in triangular implants but were not observed in rectangular grafts. The small outgrowth of tissue in the triangular type grafts is thought to be the result of union of the grafts with the dorsal surface only of the hosts, since it was also observed in cases of incomplete union in which the host ventral surface was not involved. The monophthalmic condition was found by the writer to be the result of the partial removal of one of the graft eyes during operation and the absence of any regeneration. With rectangular grafts the ability of complete takes to reorganize host tissues was in many cases equal to that of incomplete takes. Pharynges were induced posterior to the implant in grafts in the pharynx; and in the tail, induction by complete takes extended both anteriorly and posteriorly (Figs. 39, 40, 41, 67, 68, 70, 72, and 96). In fact, 1 of the

2 cases in which a pharynx was induced anterior to a graft in the pharynx level was a case of complete union (Fig. 41). This is in contrast to the findings of Santos (1931) with triangular grafts.

POLARITY

The anteroposterior polarity of cephalic grafts is shown in these experiments to be extremely labile. In cases of complete union there was little evidence that the axes of the grafts were modified except in anteroposterior grafts in the head. In these it was not possible to determine positively whether the implant retained its original axial orientation or not, but it seems likely that reversal occurred. There was no indication of lack of co-ordination of movement between host and implant or of cilia beating in opposite directions. The direction of the ciliary beat has been used by Rustia (1925) and by F. S. Miller (1937) as a criterion of the polarity of planarians and seems to be reliable.

Grafts containing any appreciable amount of free surface always showed corresponding modifications of polarity. These modifications ranged from very slight changes, in which a single photoreceptor developed in close proximity to one of the graft eyes, through cases of 90° modification with two photoreceptors developing at right angles to the original ones (Figs. 46, 47, 48, 55, 56, 58, and 59), to complete reversal in cases in which the posterior surface of the graft remained free (Figs. 30 and 31). The latter type of modification was rare because the posterior surface of the graft united more commonly than any other, but the results were consistent.

The lability of the axial organization of the most complex and highly differentiated region of a planarian is quite remarkable. Under certain conditions eyes appear, and under others they regress and disappear. On the other hand, polarity varies with the position of the free surface or surfaces. These conditions are very difficult to reconcile with a concept of polarity such as that of Harrison (1921, 1936), involving models used in stereochemistry. In order to do so, it would be necessary to develop additional theories to explain the reorientation of the hypothetical molecules with each modification in polarity. In a case such as is shown in Figures 47 and 48, three such modifications must be accounted for. In addition, the correlation between the exposed free surface and the polarity of a cephalic graft is unaccounted for by such a theory. Similar difficulties are encountered in attempting to interpret the observed modifications of polarity in these experiments with the space-lattice concept of Przibram (1921). On the other hand, the theories of Goetsch (1929) seem to be, in reality, chiefly a restatement of observed conditions rather than any constructive hypothesis which may aid in a true understanding of the significance of this general feature of organisms.

By contrast with the foregoing, the gradient concept which has a large body of experimental proof requires no additional hypothesis to account for the determination of polarity by a free surface. According to this concept, polarity is a function of the presence of a dominant region and its morphogenetic activities along the anteroposterior axis. One of the most characteristic features of the dominant region is its relatively high rate of physiological activity, and so the problem of the origin of polarity resolves itself into the origin of a region of great activity. That regenerative processes are characterized by increased physiological activity of the cells at the free surface has been demonstrated in a large number of forms. That the polarity of a planarian regenerate is independent of the nervous system of the piece is indicated in the histological studies by Flexner (1898) on *P. torva* (syn. *E. tigrina novangliae*) and by Keiller (1910) on *Planaria*

simplicissima (syn. *Curtisia foremanii*), which showed that the ganglia of the regenerate develop independently of the old nervous system. Similarly, Beyer and Child (1930) concluded from experiments on the reconstitution of lateral pieces of *E. dorocephala* that the nervous system is not an essential factor in determining the polarity of the regenerate.

Accordingly, we may suggest that the changes in polarity of the grafts resulted from activation of the cells of the free surface, which set up a new dominant region in the regenerate, and that this region gradually expanded its physiological and morphogenetic control until the entire graft-controlled tissue was repolarized. There has been little work throwing light on gradient conditions within the head, but it is possible that the slope here is very slight. The great lability of the polarity of cephalic grafts would seem to suggest this.

As was also found by L. V. Morgan (1906) and Santos (1931), the dorsoventral organization of the planarian pieces used in these experiments was not susceptible to modification by any of the situations which arose as a result of grafting. In every case the dorsal and ventral epithelia of grafts retained their peculiarities, even though, as time passed, they gradually became replaced by host tissues.

The results of these grafting experiments emphasize the fact that the tail of planarians which undergo fission is physiologically quite different from the head. It has been recognized for some time (Hyman, 1916) that the posterior, growing region of certain forms involves special conditions within the primary anteroposterior gradient. That this region is definitely polarized is shown by the fact that tails are regenerated from posterior surfaces of pieces from this level and heads always appear at the anterior surfaces. However, the experiments reported here, and those of Santos (1931) and of F. S. Miller (1937), indicate that polarization is by no means as strong posterior to the pharynx in *E. dorocephala* and *E. tigrina* as in more anterior levels, and emphasize the fact that the high physiological activity and great regenerative powers of this region should be more thoroughly investigated from a gradient point of view.

REPLACEMENT OF GRAFT BY HOST TISSUE

With few exceptions there was a gradual replacement of graft epithelium by that of the host. This has been described on page 219; and it was noted that the photoreceptors, as well as the epithelia, were replaced. Since the eyes are a part of the nervous system, this fact would suggest that the other tissues of the graft, as well as those which could be observed, probably were also involved in the replacement.

The replacement of tissues was especially rapid in small grafts which were composed largely of ganglionic tissue. On the other hand, large grafts which contained, in addition to approximately the same amount of nervous tissue, some prepharyngeal tissue retained their specific characters for a considerable longer period, and in some cases did not show any appreciable replacement of epidermal structures during the entire course of the observations. Even in these, however, the line between graft and host faded out in time and the tissues of that region took on an appearance which was intermediate between the two species.

The structure of the anterior part of a planarian seems to offer a suggestion as to the factors which determine whether a heteroplastic graft will or will not retain its specific differences. The head of a planarian consists largely of nervous and muscular elements

with very little parenchymatous tissue. It is from the latter that the more specialized cells are normally differentiated. By contrast, in the anterior prepharyngeal region nervous tissue is limited chiefly to the nerve cords; and, although the diverticula of the digestive tract occupy a much larger space than in the head, there is considerably more packing or parenchyma tissue. Curtis and Schultze (1934) have found also that there are more "formative cells" in this than in the ganglionic region.

Grafts which retained their specific characters all united with the host by only a portion of the cut surfaces. In 2 cases they were grafts in reversed dorsoventral orientation (Fig. 29), and in the others they were grafts in normal orientation which united by only one surface. Thus, it will be noted that they were cases in which the grafts, because of free anterior and lateral surfaces, were able, to a considerable extent, to regenerate lost parts. With the regeneration of the parenchymatous tissue in the prepharyngeal region they were supplied with cells of their own species with which to replace those that became senescent, and thus they retained their specific characters. On the other hand, it is suggested that the situation, in the grafts which gradually developed host characters, was that the implanted tissues contained insufficient parenchyma cells to make the necessary replacements, and that this was accomplished by host cells.

Of considerable interest in this connection are the observations on page 230. It is well known (Stoppenbrink, 1905) that starvation causes a reduction first of the reproductive system and then of the parenchyma, digestive tract, and muscles, leaving the nervous system practically unaffected over a long period. In the case shown in Figures 53 and 54 it seems likely that starvation had reached a point where the implant was almost, if not completely, devoid of parenchymatous tissue. When feeding was reinstituted, it seems likely that host parenchyma migrated into the implant, for it soon began to take on the characteristics of the host species, although during the entire period of starvation the specific characters of the two components could be recognized. That host tissues do invade the graft is indicated by Steinmann's findings (1933). Using hosts vitally stained with *Cresylechtviolett*, he observed that the digestive tract of the host invaded the graft for a considerable distance.

GRAFT MIGRATION

In the experimental section was noted the migration of cephalic grafts in the pharyngeal region. When this migration is compared with regeneration of the controls, some striking similarities are seen. When a window is cut in a planarian, the surrounding tissues contract and a more posterior level is brought into contact with a more anterior one. During regeneration new tissue forms between the two levels until they are again separated by approximately the same distance as before. Thus, in the process of regeneration the more posterior level may be said to have "migrated" back to its original level. In terms of gradients, we may say that, when two gradient levels come in contact, new tissue forms between them until the lower level is again separated from the higher by cells representing the intervening levels of physiological activity.

It might appear, at first sight, that graft migration is fundamentally the same situation excepting that the difference in physiological level between the tissues in contact is greater. New tissue would be expected to form between graft and host until the intervening levels of the axial gradient are restored. This explanation does not, however, fit all the facts, for, according to it, the reconstituting pharynx would be expected to appear at the same level as the original one, that is, in the new tissue. This occurred in

the controls, but in the experimentals in every case but one the reconstituted pharynx was to be found caudad in the old host tissue.

The indications are that the experimental procedure in some way rejuvenates the piece which is grafted. If this is the case, it would explain the hypertrophy of the grafts and graft induced tissues in comparison to the uninfluenced regions of the host. It would also interpret the observation that on one occasion, when the laboratory temperature rose suddenly over a week-end, the grafts of a number of specimens disintegrated while the hosts remained intact. The greater muscular activity of grafts would also tend to indicate a higher general physiological activity, such as is characteristic of young planarians. If this should prove to be the case, then the facts observed regarding graft migration could be interpreted on the basis of a physiologically younger axis developing at the expense of the host tissues. Two processes are involved in a case like Figures 42 and 43: first, the development of a morphogenetic field behind the graft, and second, the elimination of tissue in front of the graft. That host tissues were actually destroyed is shown by the disappearance of structures on the left side of the host head as the graft approached, while the left side of the graft gave little evidence of suffering from its proximity to another dominant region.

If the graft is placed in anteroanterior orientation, it would be expected to migrate toward its level of origin; while if in anteroposterior orientation, it would migrate away from it. This is in agreement with observations of Santos (1931) on postcephalic grafts and with those reported here.

INDUCTION AND RANGE OF DOMINANCE

The very slight inductive ability of cephalic grafts in the head is in striking contrast to grafts in the pharyngeal and tail regions. Implants in the head induced outgrowths only when there was an exposed cut surface; and the supernumerary eyes which appeared when the grafts were ectopic or in reversed anteroposterior orientation are, at best, very questionable cases of induction. Since in two of the controls supernumerary eyes developed, there is a possibility that those of the experimentals were the result of regeneration by the host under slightly abnormal conditions. The occasional auricle-like structures may also have been regenerated rather than induced.

In the pharyngeal level, cephalic grafts have greater inductive power than in the head, though less than in the tail. Short to medium postcephalic outgrowths developed, and the level at which reconstituting pharynges appeared was considerably farther posterior than in controls. Since these developed by reorganization of tissue which had not been injured in the operations, and since no control showed such development, we may say that these pharynges were graft-induced.

Placed in the tail, cephalic grafts induced reorganization cephalad as well as caudad. Santos (1929, 1931) reported the induction of pharynges in reversed polarity by grafts in postcephalic regions but did not show the condition of the digestive tracts of these cases. Feeding the animals blood has made it possible, in many of the cases described here, to observe that the digestive tracts were completely reorganized between the graft and the induced pharynges both anterior and posterior to the implant. There were in certain cases structural evidences of induction at a still greater distance from the implant. In those a second tail developed between the original host pharynx and the more anterior graft-induced one (Fig. 83). Observations on the behavior of the compounds also indicated that graft dominance extended beyond the more anterior of the induced

pharynges. During locomotion there were well-defined zones under the influence of the host and of the graft. Stimulation of the graft resulted in contraction of the host as far anterior as the induced tail. On the other hand, stimulation of the host, unless severe, had little or no visible effect upon regions posterior to the induced tail.

Inductive capacity is associated with the ability to inhibit independent differentiation by tissues in the neighborhood of the inducer. The distance along the axis that this controlling action extends has been called "range of dominance" (Child, 1924). With the dominant region anterior, the controlling action extends in a posterior direction only. However, when a dominant region is placed in the pharyngeal region or tail, it may extend its control in an anterior direction as well. It is possible to obtain a rough measure of the anterior range of dominance of a graft by decapitation of the host at various distances anterior to the implant. Data from the decapitation experiments showed that the range of graft dominance was greater in the tail than in the pharyngeal region. In the case of grafts in the pharyngeal region "inhibitory dominance" seldom extended more than 2 mm. cephalad. On the other hand, grafts in the tail usually inhibited head regeneration from a cut surface as far anterior as the host pharynx, a distance of about 4-6 mm.

ROLE OF THE NERVOUS SYSTEM IN PLANARIAN INDUCTION

The fact that the heads of planarians are largely composed of nervous tissue would suggest the possibility that this tissue was responsible in some manner for the resistance of the cephalic level to induction. If this were the case, there should be a marked reduction in host resistance when implants were made directly behind the head in the anterior prepharyngeal region. Figures 31, 32, 33, 34, 35, 36, and 37 show that this was not the case. The results of Okada and Sugino (1934) on the Japanese *P. gonocephala* are of some interest in this connection. Their reported inductions of pharynges by implants from prepharyngeal regions show that in the Japanese species ganglionic material is not necessary for the reorganization of lower levels in the gradient. Santos (1931) did not report any inductions by implants from this level in *E. dorocephala* and *E. tigrina*; but this matter probably should be reinvestigated, since it is known that pharynges will reconstitute in prepharyngeal pieces even if regeneration of a head is completely inhibited (Child, 1911c).

SUMMARY

1. A technique of operation involving rectangular transplants and ice as anesthetic gave a high percentage of takes between *E. dorocephala* and *E. tigrina*. Four hundred and twelve cases of successful implantation of cephalic grafts were studied. Of these, 105 were in the head, 113 in the pharyngeal level, and 174 in the tail. Twenty were placed in the anterior prepharyngeal region for comparative purposes.

2. Union between the digestive tract of the implant and host was necessary for persistence and growth but not for regeneration of the graft. In all levels a free surface was necessary for regeneration by the transplant and determined its anteroposterior axis. Modifications of polarity up to 180° were observed. When union between graft and host was complete, there was no graft regeneration, and polarity apparently remained unmodified. Dorsoventrality of the grafts in all cases remained unchanged during the entire period of observation.

3. In most cases there was a gradual centripetal replacement of graft epithelia by

those of the host. There was some evidence that tissues other than the epithelia were also involved. When the amount of union between graft and host was slight, this replacement did not take place.

4. Cephalic grafts in the head commonly united with the hosts by all surfaces and became incorporated in them. Their epithelia and photoreceptors eventually were replaced by host tissue.

5. When the anteroposterior axis of hosts and grafts in the head region did not coincide, whether by accident or as a result of implantation in anteroposterior orientation, supernumerary photoreceptors occurred.

6. When grafts in the head did not unite completely, very short postcephalic outgrowths developed; and often from the free edge of the host window a more or less complete secondary head appeared.

7. In grafts in dorsoventral orientation there was more or less formation of new tissue between graft and host. In some cases this resulted in the development of additional heads, one surface of which developed from the implant and the other from the host.

8. Grafts in the nonganglionic, anterior prepharyngeal region showed very little more ability to induce than did those in the head, indicating that resistance to induction is not dependent upon the amount of nervous tissue present.

9. Grafts in the pharyngeal region induced larger postcephalic outgrowths than did those in the head. These were flat in cases of incomplete union but tubular in complete or nearly complete takes. A pharynx was induced posterior to the level of implantation in almost every case. In two cases a pharynx reversed in polarity was induced anterior to the graft.

10. A gradual migration was noted in a number of grafts in the pharyngeal region. This was most evident during inanition and was characterized by the appearance of new tissue behind the graft and the disappearance of host tissue anterior to it.

11. Cephalic implants in the tail region induced the largest outgrowths of tissue and the greatest reorganization of the host. This included reorganization of the two branches of the postpharyngeal digestive tract into a single prepharyngeal branch, development of pharynges anterior and posterior to the implant, and in a few cases a supernumerary tail between the pharynx of the host and that induced anterior to the graft.

12. Triangular cephalic grafts containing more anterior levels but less actual tissue than the rectangular implants showed greater inductive capacities than the latter.

13. Decapitation of the host anterior to the implants showed that the range of dominance of grafts in the pharyngeal region usually did not extend more than about 2 mm. cephalad. In the tail region, graft dominance usually extended about to the posterior tip of the host pharynx.

14. Double forms were developed from the posterior fission pieces of grafts with two tails. These possessed two pharynges, doubled prepharyngeal branches of the digestive tract, and more or less doubled postpharyngeal branches. Perpetuation of these by fission occurred, but they certainly could not be called hereditary modifications.

15. The gradient in host resistance to induction was strikingly illustrated in cases in which grafts were simultaneously implanted in different levels of the same host.

16. In the discussion the following topics are treated: (a) triangular versus rectangular grafts, (b) polarity, (c) replacement of graft by host tissue, (d) graft migration, (e) induction and range of dominance, and (f) role of the nervous system in planarian induction.

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PLATE I

PHOTOGRAPHS OF LIVING ANIMALS

FIGS. 28-30, 33, 71, 83-86, 89-91, AND 96.—*E. dorotocephala* into *E. tigrina novangliae*.
FIGS. 31 AND 32.—*E. tigrina novangliae* into *E. dorotocephala*.

FIG. 1.—*E. dorotocephala*.

FIG. 2.—*E. tigrina*.

FIG. 3.—*E. tigrina novangliae* possessing mid-dorsal light stripe, type used in these experiments. Note pharynx (*P*).

GRAFTS IN THE HEAD REGION

FIG. 28.—DVAA. Union complete. 273 days. Graft epithelia replaced by host tissue. Dorsoventral axis unaltered and no regeneration of host eyes. Same specimen shown in Fig. 27, 1 week after operation.

FIG. 29.—DVAA. Union by posterior part of dorsal surface only. 280 days. Graft (*G*) has regenerated head, tail, and functional pharynx. Species specificities kept during 10 months of observation.

FIG. 30.—DVAP. Posterior surface of graft failed to unite with host. 21 days. No host head developed during 133 days of observation.

GRAFTS IN THE ANTERIOR PREPHARYNGEAL REGION

FIG. 31.—DDAA. Union complete. 175 days. Two auricle-like structures developed lateral to implanted eyes. Graft epithelia replaced by host tissues.

FIG. 32.—DDAA. Anterior surface of implant failed to unite with host. 180 days. Host tissue disintegrated to right of graft. Epithelia, head shape, and cephalic lobes of implant (*G*) typical of host rather than graft.

GRAFTS IN THE TAIL

FIG. 71.—DDAA. Union complete. 252 days. Note tubular, graft-induced outgrowth with pharynx (*P*) posterior to, and with pharynx (*P'*) and very small projection anterior to, implantation level.

FIG. 83.—DDAA. Union by graft posterior surface only. 133 days. Reorganization of digestive tract with induction of pharynx (*P*) anterior as well as posterior to implant. Note tail (*T*) between host pharynx and anterior graft-induced one.

FIG. 84.—DVAA. Union by dorsal surfaces only. 21 days. Host fissioned during first week. Outgrowth of tissue does not involve ventral surface of host. Intestinal diverticula of graft failed to unite with those of host. Implant subsequently regressed and finally disintegrated.

FIG. 85.—DVAP. Union by posterior and left edge of graft. 28 days. Host window tore apart at posterior angles 1 week after operation, and sides have developed into tails. Unpigmented areas of three graft eyes (one *R* regenerated) seen through ventral surface.

FIG. 86.—DVAP. Union complete. 70 days. Host fissioned immediately behind implant (*G*), and regenerated head (*R*) instead of tail opposite that of graft. Both share common ventral surfaces and pharynx (*P*) with reversed polarity.

FIGS. 89 AND 90.—DDAP. 56 and 70 days, respectively. Anterior surface of graft failed to unite with host. Window in host tore apart posterior to implant. FIG. 89.—Postcephalic outgrowth and pharynx with reversed polarity induced by graft. Small tail-like appendage developed from window. Graft epithelia replaced by host tissues. FIG. 90.—Photographed after feeding blood to show pharynxes (*P*).

FIG. 91.—DDAP. Union by graft posterior surface. 154 days. Fission piece. Tail and pharynx (*P*) with reversed orientation developed in host anterior to implant. Fusion of tails along lateral surfaces in progress.

FIG. 96.—Two grafts (*G*). Both DDAA, union complete. 35 days. Teratophthalmic head regenerated on host after section 2 mm. anterior to first graft. Note extent of control of each head caudad and cephalad, and reorganization of digestive tract and induction of pharynx (*P*) anterior to second graft.

PLATE I

